Thesis for the degree of D.Sc.

SYNTHESIS OF QUINOLINE COMPOUNDS OF POSSIBLE THERAPEUTIC VALUE.

PART I.

4:5-BENZ-β-CARBOLINE AND ITS DERIVATIVES.

PART II.

QUINOLINE COMPOUNDS CONTAINING ARSENIC.

by

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C O N T E N T S.

PART I.

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PART II.

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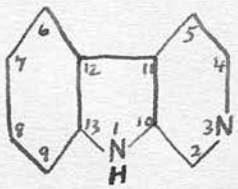
APPENDIX.

Eleven reprints embodying research work not dealt with in this thesis.



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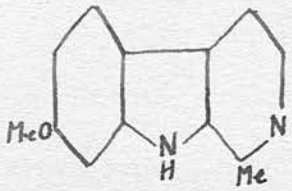

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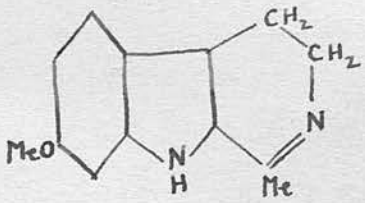

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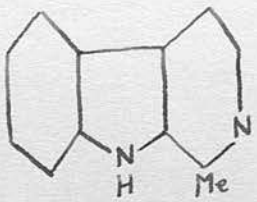


IV

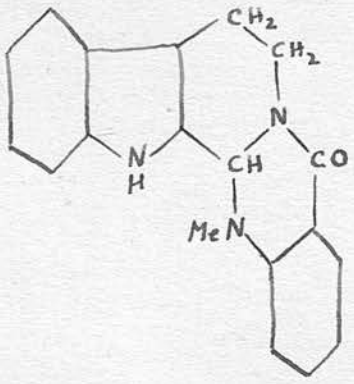
PART I.

I. Synthesis of 4:5-Benz-β-carboline and its Derivatives.

It has been shown within recent years that certain naturally occurring alkaloids are derivatives of the aromatic heterocyclic base (I) to which the name β-carboline* has been assigned. Thus the researches of W.H. Perkin, jun. and R. Robinson and their collaborators have demonstrated that harmine** and harmaline, the two principal alkaloids obtained from the seeds of Peganum harmala, possess the configurations II and III respectively (compare J., 1912, 101, 1775; 1913, 103, 1973; 1919, 115, 933, 967; 1921, 119, 1602, et seq.), whilst Späth (Monatsh., 1919, 40, 351; 1920, 41, 297) has shown that aribine and loturine, two alkaloids found in Araribra rubra Mart. and Symplocosa racemosa respectively, are identical with harman (demethoxyharmine) (IV), which can readily be obtained from harmine. The alkaloids evodiamine and/

* The nomenclature and numbering of the carboline nucleus adopted in this section of the present thesis are based on the suggestion of Gulland, Robinson, Scott and Thornley (J., 1929, 2926).

** It has been claimed by several investigators that the alkaloids banisterine, telepathine and yageine are identical with harmine (compare Elger, Helv.Chim. Acta., 1928, 11, 162; Kreitmair, Mercks Jahresber., 1929, 42, 5).



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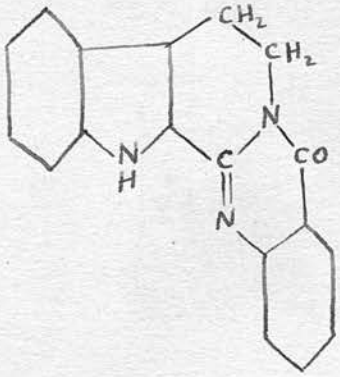

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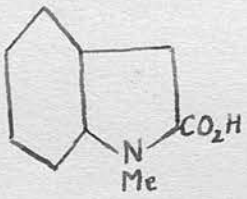

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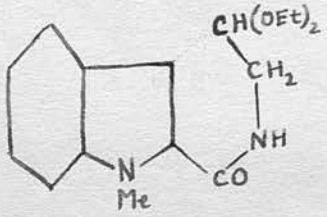

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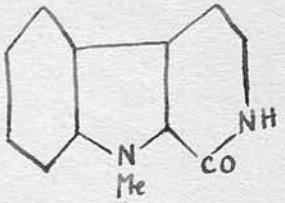

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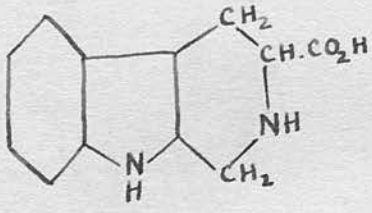
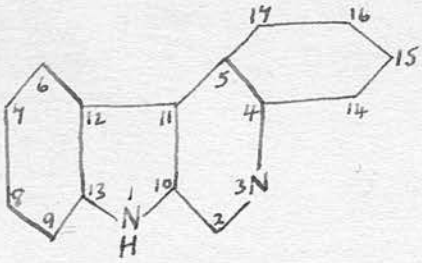


X

and rutaecarpine, which are obtained from the fruit of Evodia rutaecarpa, have also been shown to be derivatives of β-carboline (I) and to possess structures V and VI respectively (compare Kermack, Perkin and Robinson, J., 1921, 119, 1615; Asahina, Manske and Robinson, J., 1927, 1708; Asahina and Ohta, Ber., 1928, 61, 319; J. Pharm. Soc. Japan, 1928, 48, 51).

The above alkaloids have been synthesised in several ways and the methods used involve the preparation of indole derivatives containing a suitable side chain in the 2- or 3-position (compare Manske, Perkin and Robinson, J., 1927, 1; Späth and Lederer, Ber., 1930, 63, 120; Akabori and Saito, Ber., 1930, 63, 2245).

β-Carboline (norharman) (I), the parent base of all the above alkaloids, has been synthesised by Kermack, Perkin and Robinson (J., 1921, 119, 1602) in two different ways. In the first method of synthesis 1-methylindole-2-carboxylic acid (VII) was converted through its chloride into 1-methylindole--2-carboxyacetalylamide (VIII) which, on treatment with alcoholic hydrogen chloride, gave 2-keto-1-methyl--2:3-dihydro-β-carboline (IX) from which β-carboline was obtained on distillation with zinc dust. The second method of synthesis consisted in the condensation of tryptophane with formaldehyde in presence of/



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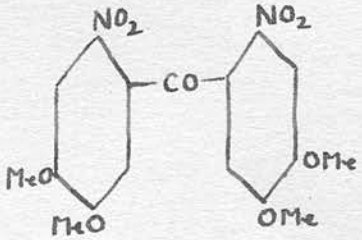

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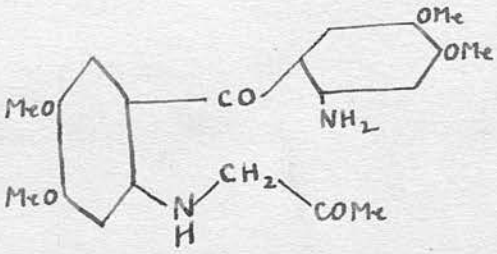

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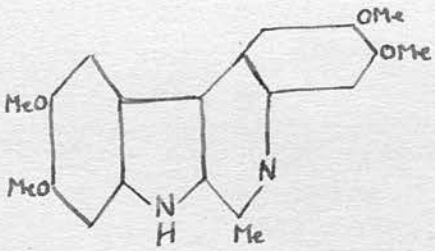

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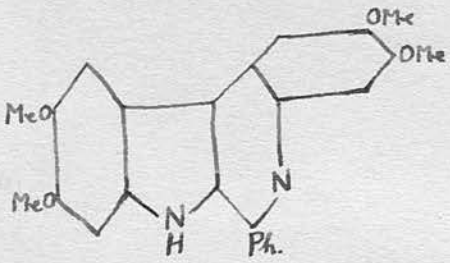

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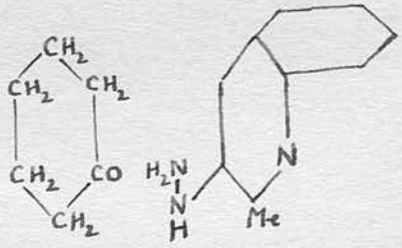
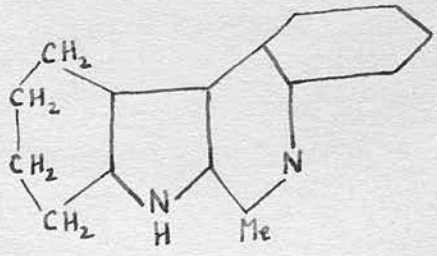


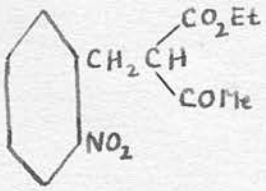
XVI XVII



XVIII

of sulphuric acid followed by oxidation of the product (X) (compare also Späth and Lederer, Ber., 1930, 63, 2102).

This section of the present thesis is devoted to an investigation of the methods for the synthesis of the analogous 4:5-benz-β-carboline (XI) and its derivatives. The following three derivatives of this base have already been prepared, namely 7:8:15:16-tetramethoxy-2-methyl-4:5-benz-β-carboline (methyldiveratroharmyrine) (XIV), 7:8:15:16-tetramethoxy-2-phenyl-4:5-benz-β-carboline (phenyldiveratroharmyrine) (XV) (Lawson, Perkin and Robinson, J., 1924, 125, 626), and 2-methyl-6:7:8:9-tetrahydro-4:5-benz-β-carboline (XVIII) (Robinson and Robinson, J., 1924, 125, 827). The synthesis of compound XIV may be briefly described as follows. 6:6'-Dinitro-3:4:3':4'-tetramethoxybenzophenone (XII) was reduced to the corresponding diaminoveratrone which was then condensed in acetic acid with bromoacetone to yield compound XIII from which the carboline was obtained on treatment with alkali. Compound XV was synthesised in the same way, ω-bromoacetophenone being used instead of bromoacetone. For the synthesis of compound XVIII, 3-amino-2-methylquinoline was diazotised, the product reduced to 3-hydrazino-2-methylquinoline (XVII), and the latter condensed with cyclohexanone/



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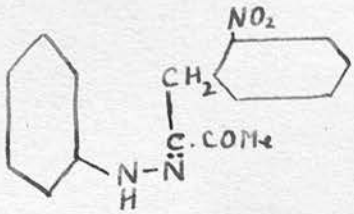

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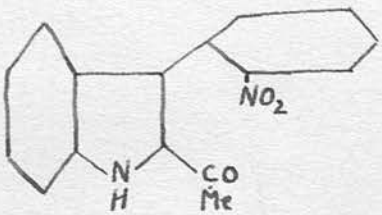
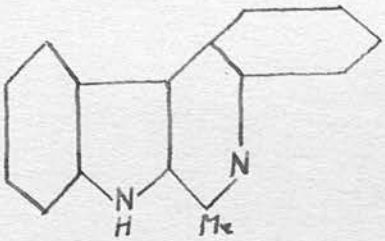


XXI



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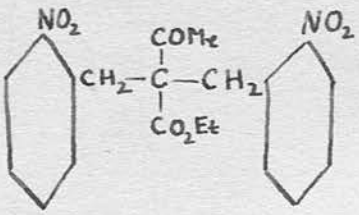

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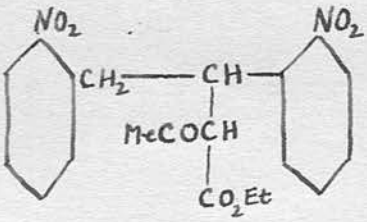
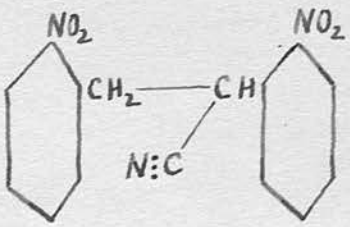


XXIV

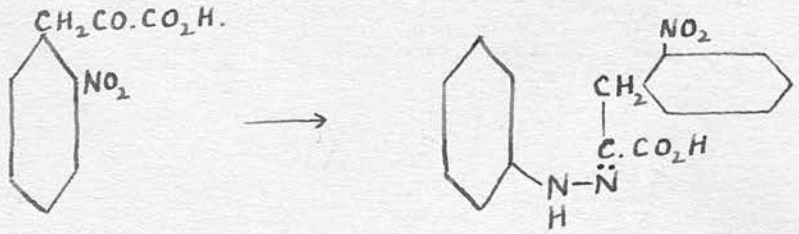


XXV

cyclohexanone (XVI). It is, however, difficult to effect the synthesis of 4:5-benz-β-carboline itself by either of the above methods. The 2:2'-diaminobenzophenone required for the application of the first method of synthesis cannot be readily obtained, whilst in the case of the second method the methyl group attached to the quinoline nucleus in the 2-position is necessary to ensure that cyclisation will result in the production of an unambiguous compound. Further, the product obtained by this latter method is the tetrahydro derivative and not the aromatic base.

Two distinct schemes for the synthesis of 4:5-benz-β-carboline and its derivatives have been investigated. In the first of these it was proposed to condense o-nitrobenzyl chloride with ethyl acetoacetate and so obtain ethyl o-nitrobenzylacetoacetate (XIX). It was thought that this compound, after reaction with benzenediazonium chloride so as to yield the phenylhydrazone of o-nitrophenylbutane-β-γ-dione (XX), might be convertible into 2-acetyl-3-o-nitrophenylindole (XXI). This product on reduction should lose water and thus yield 2-methyl-4:5-benz-β-carboline (4:5-benzharman) (XXII)*. Experiments were carried out under various/

* A similar method has been used very effectively by Manske, Perkin and Robinson (J., 1927, 1) in the synthesis of harmaline.



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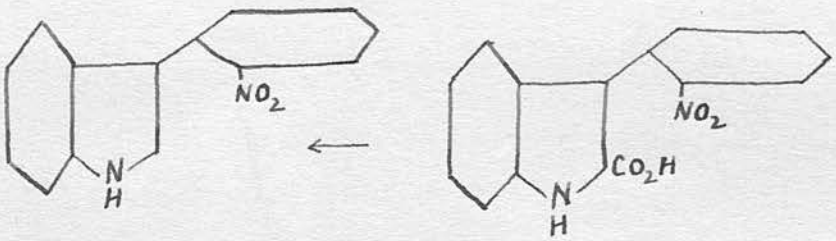
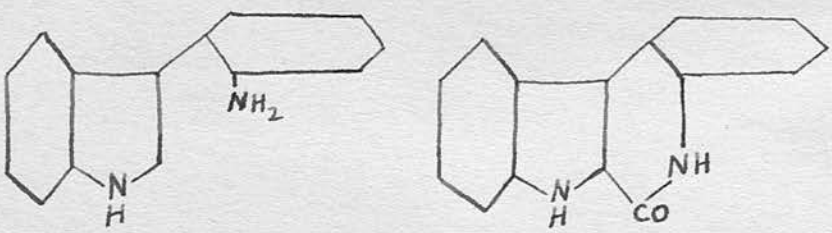
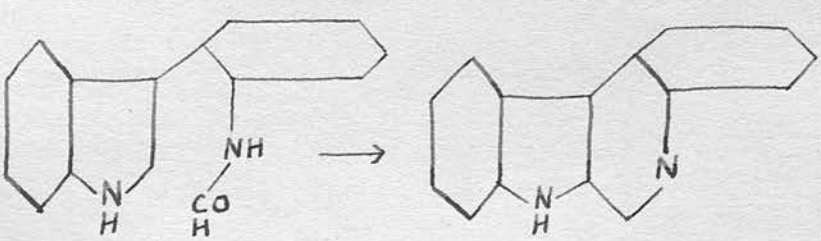


XXXI XXVIII



XXXII XXIX



XXXIII XXX

various conditions with the aim of obtaining the desired ethyl o-nitrobenzylacetoacetate. It was found, however, that besides the compound, m.p. 103°, described by Reissert (Ber., 1896, 29, 637), and formulated by him as XXIII , another compound, m.p. 183°, could also be isolated. This compound, apparently an isomeride of Reissert's substance, was insoluble in aqueous sodium carbonate but soluble in sodium hydroxide solution and it is difficult to formulate it otherwise than as XXIV . The formation of this compound may be compared with that of α-cyano-α-β-di-o-nitrophenylethane (XXV) by the action of potassium cyanide on o-nitrobenzyl chloride (compare Gabriel and Eschenbach, Ber., 1897, 30, 3018).

Since ethyl o-nitrobenzylacetoacetate could not be obtained in the above way, attention was directed to an altogether different process for the synthesis of 4:5-benz-β-carboline and its derivatives. This scheme is diagrammatically represented on the opposite page. For these syntheses o-nitrophenylpyruvic acid (XXVI) was required in large quantities and so a number of experiments were carried out in order to ascertain the best method for its preparation. The original method described by Reissert (Ber., 1897, 30, 1036) consisted in condensing o-nitrotoluene and ethyl oxalate by means of alcoholic sodium ethoxide. This method, however, gave yields of less than 50 per cent., and/

and it was found that the working up of the product so as to obtain the pure crystalline acid was very laborious and sometimes uncertain. Blaikie and Perkin (J., 1924, 125, 310) have shown that certain methoxy derivatives of o-nitrotoluene can be condensed readily with ethyl oxalate in ethereal solution in the presence of potassium ethoxide. It has now been found that when special precautions are taken this method can be utilised for the preparation of o-nitrophenylpyruvic acid in excellent yield and in a state of high purity.

The phenylhydrazone of o-nitrophenylpyruvic acid (XXVII), which has already been described by Reissert (Ber., 1897, 30, 1038), readily underwent the Fischer indole condensation when its boiling alcoholic solution was saturated with dry hydrogen chloride. The resulting ethyl 3-o-nitrophenylindole-2-carboxylate was hydrolysed by means of dilute alcoholic potassium hydroxide and converted through the potassium salt into the free acid (XXVIII), which was readily obtained in a pure condition. The alkaline mother liquors from the preparation of the potassium salt contained a neutral compound, which proved to be 3-o-nitrophenylindole (XXXI). It was formed, apparently, during the indole cyclisation by loss of carbon dioxide and it was observed that the amount of 3-o-nitrophenylindole produced varied with the duration of boiling during the saturation with hydrogen chloride;

in/

in some experiments a large yield of 3-o-nitrophenylindole was obtained accompanied by very little acid. Attempts to increase the yield of the acid by carrying out the saturation of the alcoholic solution with hydrogen chloride at a temperature of 50° did not lead to the desired result since the potassium salt isolated after boiling with alcoholic potassium hydroxide consisted mainly of that of the unchanged phenylhydrazone. In order to prepare 3-o-nitrophenylindole in quantity, efforts were made to eliminate the carboxyl group from 3-o-nitrophenylindole-2-carboxylic acid. When the calcium salt was heated under various conditions, charring took place and, when the acid itself was heated slightly above its melting point, decomposition occurred but only a comparatively small yield of 3-o-nitrophenylindole was obtained. It was found, however, that when the ammonium salt of the acid was heated at 270-280° smooth decomposition occurred and 3-o-nitrophenylindole was isolated from the product in very good yield.

When 3-o-nitrophenylindole-2-carboxylic acid (XXVIII) was treated with zinc dust in acetic acid solution, reduction of the nitro to the amino group took place but ring-closure also occurred with very great ease, and the sparingly soluble white product which separated proved to be 2-keto-2:3-dihydro-4:5-benz-β-carboline (XXIX), a compound which exhibits

a/

a blue fluorescence in acid solutions. The facility with which ring-closure takes place was shown by the fact that, in an experiment in which the reduction was carried out by means of ferrous sulphate and ammonium hydroxide so that the solution remained alkaline, no product of the reduction could be detected in the filtrate from the ferric oxide, but the product was obtained as the carboline derivative after dissolution of the ferric oxide in dilute hydrochloric acid. This experiment clearly indicates that ring closure takes place even in alkaline solution.

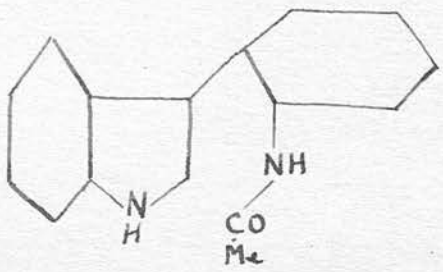
2-Keto-2:3-dihydro-4:5-benz-β-carboline may be compared with 2-keto-3-methyl-2:3-dihydro-β-carboline and other similar derivatives previously described (Kermack, Perkin and Robinson, J., 1922, 121, 1886; Blaikie and Perkin, loc. cit, p.319). Like these compounds it exhibits a strong blue fluorescence in very dilute acid solutions but it is much less soluble in the usual solvents.

The conversion of 2-keto-2:3-dihydro-4:5-benz-β-carboline (XXIX) into 4:5-benz-β-carboline (XXX) presented difficulties and only when it was reduced with zinc dust in a current of hydrogen could a small amount of the base be obtained. This method was obviously not adapted for the preparation of the compound in quantity. It was found, however, that this/

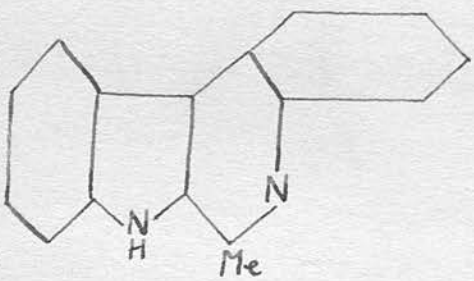
this and other 4:5-benz-β-carboline bases could be obtained, as described below, from 3-o-nitrophenylindole (XXXI) the preparation of which has already been described (see pp.8-9).

The reduction of 3-o-nitrophenylindole to the corresponding amino-compound proceeds satisfactorily only under special conditions. When it was reduced in acetic acid solution with zinc dust, a non-basic substance, m.p. about 265°, was obtained, the constitution of which has not yet been elucidated. Owing, apparently, to the insolubility of the nitrophenylindole in water, reduction with tin and aqueous hydrochloric acid or with stannous chloride solution did not take place. When the compound was dissolved in alcohol containing hydrochloric acid and the boiling solution treated with tin, reduction occurred but very little or none of the aminophenylindole could be isolated from the reaction product. It appeared that this difficulty was due to the fact that the base forms highly insoluble compounds both with stannic salts and with hydrochloric acid and that the former are not readily decomposed by alkali. Ultimately it was found that 3-o-aminophenylindole (XXXII) could be readily obtained in good yield by reduction of the nitro compound in alcoholic solution with iron and hydrochloric acid according to the method described by West (J., 1925, 127, 494).

When/



XXXIV



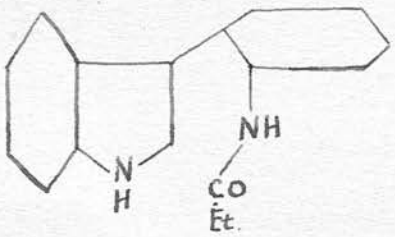
XXXV

When 3-o-aminophenylindole was refluxed with anhydrous formic acid, 3-o-formamidophenylindole (XXXIII) was obtained mixed with a small quantity of a fluorescent base, evidently 4:5-benz-β-carboline (XXX). The crude formyl derivative was dissolved in boiling toluene and treated with phosphoryl chloride. Under these conditions ring closure took place with the elimination of water and the formation of 4:5-benz-β-carboline (XXX). This interesting base, the parent substance of this series of compounds and of the diveratroharmyrine derivatives prepared by Lawson, Perkin and Robinson (loc. cit.) is in many respects similar to β-carboline itself, and exhibits an intense bluish-green fluorescence in acid solutions. This compound proved to be identical with that obtained in extremely small yield by the zinc dust distillation of 2-keto-2:3-dihydro-4:5-benz-β-carboline (see p.10).

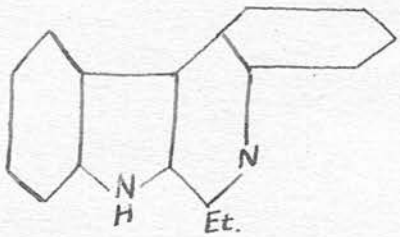
Various homologues of 4:5-benz-β-carboline were prepared in a similar manner as described below.

3-o-Aminophenylindole was readily acetylated when it was boiled in acetic anhydride solution. The product, 3-o-acetamidophenylindole (XXXIV), was obtained in excellent yield. A number of experiments were carried out with this compound in order to obtain some knowledge as to the ease with which ring closure takes/

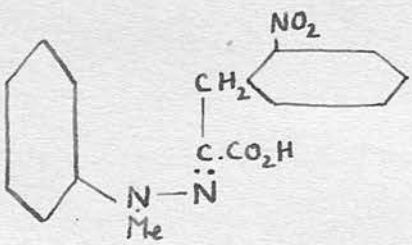
takes place. When it was boiled for a long time with acetic anhydride, with or without the addition of zinc chloride, so as to form 2-methyl-4:5-benz-β-carboline (XXXV), ring closure appeared to take place to some slight extent as evidenced by the appearance of a fluorescence, but in any case the yield was so small that none of the desired compound could be isolated. Saturation of the solution of the acetyl derivative in alcohol with hydrogen chloride, or addition of phosphorus pentoxide to the solution in boiling benzene were even less effective as in neither case was there even a development of the fluorescence. However, when the solution in dry toluene was boiled with phosphoryl chloride, ring closure readily took place as in the case of the formyl derivative, and the product proved to be the expected 2-methyl-4:5-benz-β-carboline (XXXV). This compound has many points of resemblance to harman (IV). It gives none of the usual indole reactions but exhibits a very marked blue fluorescence in acid solutions. It appears to be a much weaker base than harman, and it shows no tendency to absorb carbon dioxide from the atmosphere. In this respect it is interesting to compare it with methyldiveratroharmyrine (XIV) described by Lawson, Perkin and Robinson (loc. cit.); this latter compound is apparently so strongly basic that special/



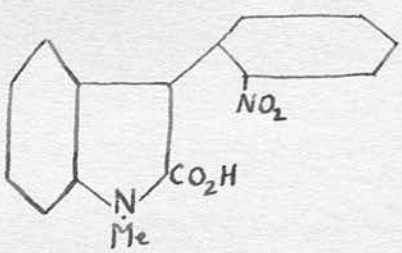
XXXVI



XXXVII



XXXVIII

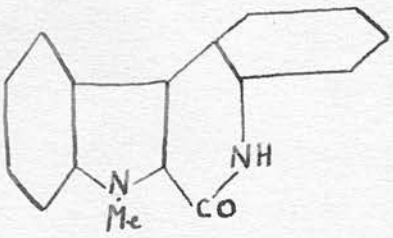


XXXIX

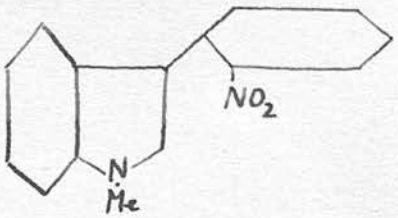
special precautions had to be taken to exclude carbon dioxide during its preparation. It is also interesting to note that 2-methyl-4:5-benz-β-carboline does not exhibit any tendency, when precipitated from solution in the cold, to form a gelatinous mass like that characteristic of the above tetramethoxy compound. Thus it appears that this peculiar property of the latter compound does not depend merely on the nuclear structure.

In the same way, 3-o-propionamidophenylindole (XXXVI) was converted by the action of phosphoryl chloride in presence of toluene into 2-ethyl-4:5-benz-β-carboline (XXXVII). Apart from a characteristic tendency to combine with and retain solvents, this compound closely resembles 4:5-benz-β-carboline and its 2-methyl derivative, which have been described above, and, in particular, solutions of its salts exhibit a very brilliant bluish-green fluorescence.

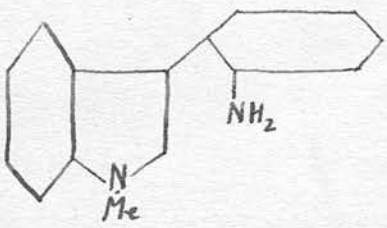
o-Nitrophenylpyruvic acid phenylmethylhydrazone (XXXVIII) underwent the Fischer indole condensation when acted upon with dilute hydrochloric acid although o-nitrophenylpyruvic acid phenylhydrazone (XXVII) showed no tendency to cyclise under these conditions. This result is in keeping with the general experience that hydrazones prepared from phenylmethylhydrazine undergo indole cyclisation with much greater ease than/



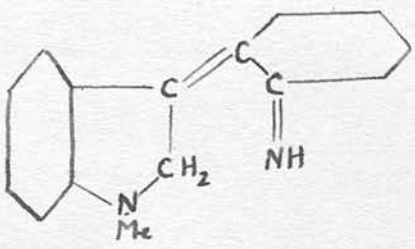
XL



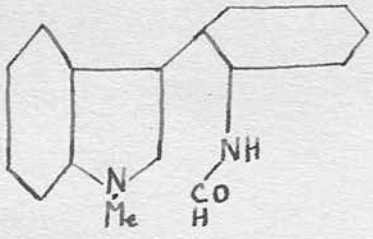
XLI



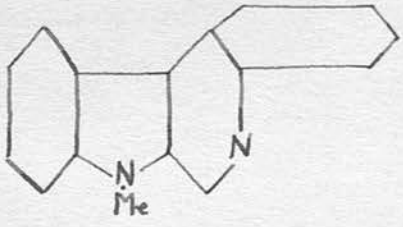
XLII



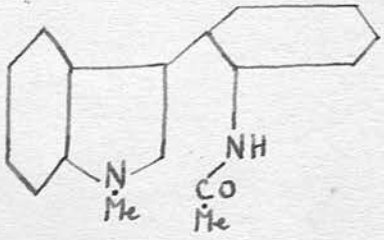
XLIII



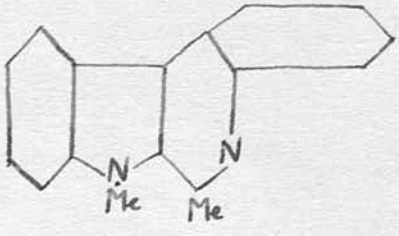
XLIV



XLV



XLVI



XLVII

than the corresponding ones obtained from phenylhydrazine. The product of the reaction was 3-o-nitrophenyl-1-methylindole-2-carboxylic acid (XXXIX), which is very similar in its properties to 3-o-nitrophenylindole-2-carboxylic acid (XXVIII). Thus, when it was reacted upon with zinc dust and acetic acid, it yielded 2-keto-1-methyl-2:3-dihydro-4:5-benz-β-carboline (XL), and when heated to 250° it decomposed with evolution of carbon dioxide and formation of 3-o-nitrophenyl-1-methylindole (XLI). This nitro-derivative was reduced in alcoholic solution to the corresponding amino-compound by means of iron filings and hydrochloric acid. 3-o-Aminophenyl-1-methylindole is peculiar in that it possesses an old-gold colour, and is only sparingly soluble in alcohol and ether, whereas 3-o-aminophenylindole itself is pure white and is readily soluble in these solvents. Further, the N-methyl derivative melts at 47° above the parent base, whereas in all other cases in this series the melting point of the methyl derivative is lower than that of the compound from which it is derived. These facts suggest that 3-o-aminophenyl-1-methylindole may possess a quinonoid structure and that its formula may be XLIII rather than XLII .

When 3-o-aminophenyl-1-methylindole was refluxed with anhydrous formic acid, 3-o-formamidophenyl-1-methylindole (XLIV) was obtained. The crude formyl/

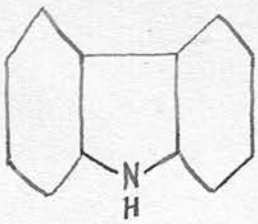
formyl derivative was readily converted into 1-methyl-4:5-benz-β-carboline (XLV) on treatment with phosphoryl chloride in boiling toluene solution, ring closure taking place with great ease. In the same way 3-o-acetamidophenyl-1-methylindole (XLVI) was converted into 1:2-dimethyl-4:5-benz-β-carboline (XLVII). These carboline derivatives closely resemble 4:5-benz-β-carboline (XXX) and its 2-methyl and 2-ethyl derivatives (XXXV and XXXVII), and fluoresce strongly in acid solutions.

A consideration of the formula of 3-o-nitrophenylindole-2-carboxylic acid (XXVIII) suggests the possibility that the free rotation of the o-nitrophenyl group might be inhibited by the carboxyl group on the one hand and the projecting apex of the benzenoid ring on the other. It appears probable that the optical activity of certain diphenyl derivatives is occasioned by the absence of free rotation due to steric hindrance (compare Christie and Kenner, J., 1922, 121, 614, et seq.), and it was therefore desirable to ascertain whether 3-o-nitrophenylindole-2-carboxylic acid could be resolved. The brucine salt was accordingly prepared, and this well-defined compound was recrystallised from alcohol but no evidence of resolution was observed. The acid, when liberated carefully in the cold from its brucine salt, proved to be quite inactive. It seems,/

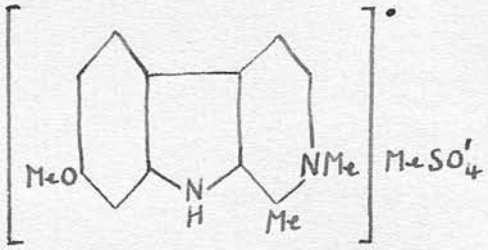
seems, therefore, that the projecting apex of the benzenoid ring adjacent to the point of attachment of the o-nitrophenyl group is, in this case at least, insufficient to prevent free rotation.

Since Gunn and Marshall (Proc. Roy. Soc., Edinburgh, 1920, 40, 145) have found that both harmine and harmaline possess definite chemotherapeutic actions in certain types of malaria, it was considered of interest to ascertain the therapeutic values of the closely related 4:5-benz-β-carboline and its derivatives with respect to this disease. These compounds have been tested by the Chemotherapy Committee of the Medical Research Council on bird malaria; the results however were negative.

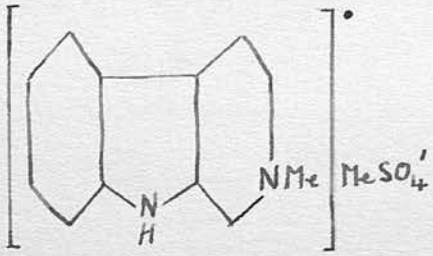
The above 4:5-benz-β-carboline compounds have also been tested by Professor Hesse, Breslau, in respect of their chemotherapeutic actions in tuberculosis. The results in this case also were negative.



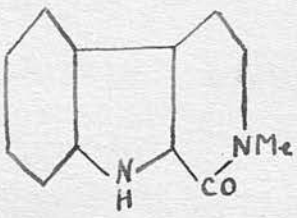
XLVIII



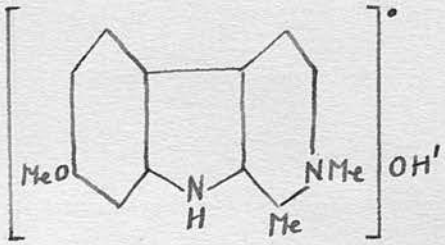
XLIX



L



LI



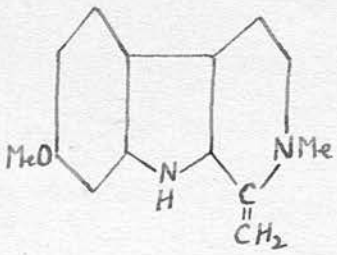
LII

II. The Constitution of Methosulphates and of Anhydronium Bases prepared from β-Carboline Derivatives.

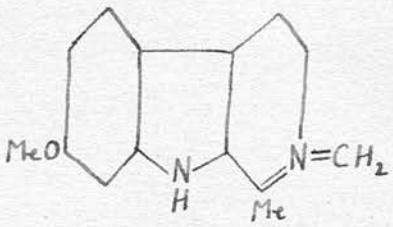
The close relationship which exists between the constitutions of harmine (II) and carbazole (XLVIII) and the fact that the nitrogen atom in carbazole is practically devoid of basic character led Perkin and Robinson (J., 1919, 115, 941) to the view that the pyridine nitrogen atom is responsible for the salt-forming properties of harmine. They accordingly assigned to harmine methosulphate, formed by the addition of methyl sulphate to harmine, the structure XLIX. This formula is confirmed by the oxidation of the analogous β-carboline methosulphate (L) to 2-keto-3-methyl-2:3-dihydro-β-carboline (LI), a compound which has been prepared synthetically in such a way as to leave no doubt as to its constitution (Kermack, Perkin and Robinson (J., 1922, 121, 1877).

Now when a solution of harmine methosulphate is treated with aqueous sodium hydroxide, the methohydroxide (LII) produced, when heated at 100°, readily loses a molecule of water with the formation of a new base, methylharmine (Perkin and Robinson, loc. cit., p. 942). There are three possible ways in which water

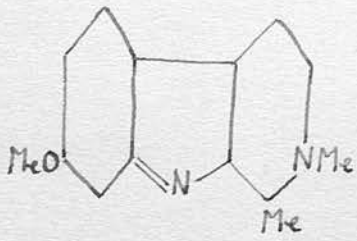
can/



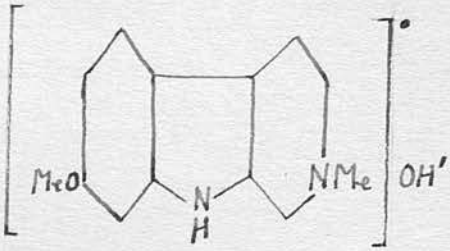
LIII



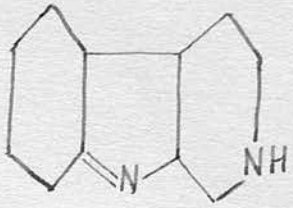
LIV



LV

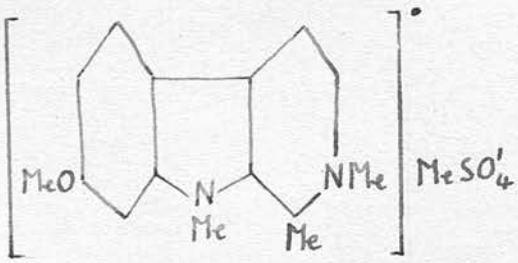


LVI

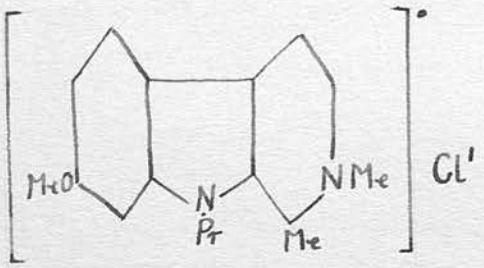


LVII

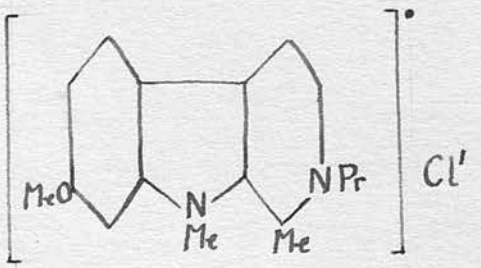
can be eliminated from harmine methohydroxide with production of methylharmine, namely by combination of the hydroxyl group with (a) a hydrogen atom of the C-methyl group, (b) a hydrogen atom of the N-methyl group, (c) the hydrogen atom of the imino-group in the pyrrole nucleus: these give rise to formulae LIII, LIV and LV respectively for methylharmine. Perkin and Robinson (loc. cit.) definitely excluded formula LIII since norharmine methohydroxide (LVI), which contains no C-methyl group and is formed from norharmine methosulphate by the action of sodium hydroxide, can be readily converted into methylnorharmine which entirely resembles methylharmine in its properties. They also ruled out formula LIV on the grounds that, apart from the unusual type of quinquevalent nitrogen atom which this formula possesses, if it were a true representation of the structure of methylharmine then it would be expected that similar methylpyridines and methylquinolines could be prepared from pyridine and quinoline methohydroxides in the same way. Perkin and Robinson (loc. cit.) therefore assigned to methylharmine the formula LV, which is the only other possibility. This formula represents methylharmine as being a derivative of β-ψ-carboline (LVII) which differs from β-carboline (I) in having a hydrogen atom attached to the pyridine nitrogen atom instead/



LVIII



LIX



LX

instead of to the pyrrole nitrogen atom.

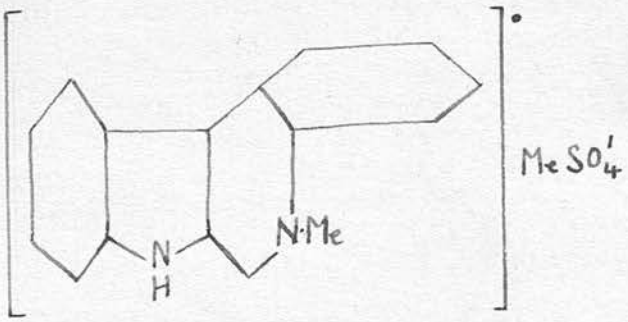
Now methylharmine readily reacts with another molecule of methyl sulphate to yield the salt methylharmine methosulphate. This methosulphate, on treatment with alkali, gives rise to the corresponding methohydroxide which, as would be expected, shows no tendency to lose a molecule of water and to form a new base. Perkin and Robinson have assigned to methylharmine methosulphate the constitution LVIII, methyl sulphate being added to methylharmine at the pyrrole nitrogen atom. This nitrogen atom, then, appears to acquire basic properties in methylharmine as the result of the rearrangement of co-valencies in order to accommodate the methyl group now present on the pyridine nitrogen atom.

In order to obtain evidence in support of the above view of the constitution of the anhydronium base (LV) and of methylharmine methosulphate (LVIII), Kermack, Perkin and Robinson (loc. cit.) prepared isomeric methylpropylharmine chlorides by introducing the alkyl groups in a different order. By the addition of propyl iodide to methylharmine, followed by conversion to the chloride, the compound methylharmine propochloride was obtained which according to the theory would possess formula LIX. On the other hand the compound formed by the addition of methyl iodide/

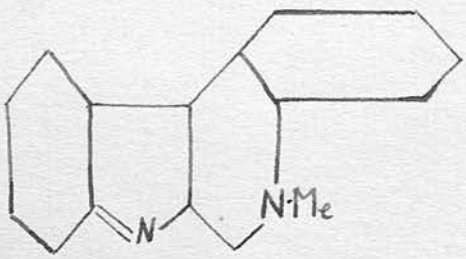
iodide to propylharmine (which itself is formed by the action of alkali on harmine propoiodide), when converted into the chloride, ought to be represented by formula LX, and, therefore, ought not to be identical with the other (LIX). These two compounds, although similar in many of their properties, proved not to be identical, and so the theory is confirmed. It is evident that if the two alkyl groups added on in both cases to the same nitrogen atom these two methyl-propylharmine chlorides would probably prove to be identical.

These facts lend strong support to the above view of the constitution of these anhydronium bases, but this evidence is not quite conclusive inasmuch as it is conceivable that the addition of the alkyl salts to the anhydronium base might take place in such a way that the alkyl group did not attach itself to either nitrogen atom. This possibility, although rather unlikely, must be considered and, therefore, direct evidence as to the constitution of the anhydronium bases in the carboline series appears desirable. Definite evidence upon this point would obviously be of importance with reference to the general problem of the structure of heterocyclic polynuclear bases.

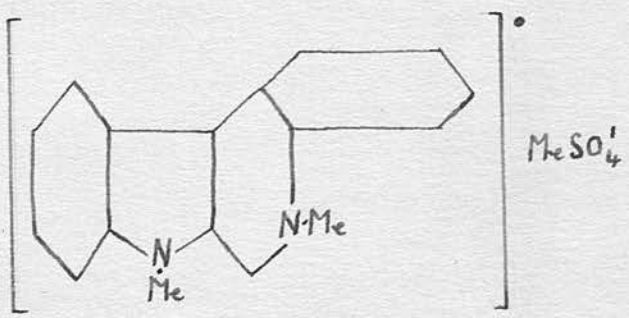
4:5-Benz-β-carboline (XXX) and its homologues have/



LXI



LXII



LXIII

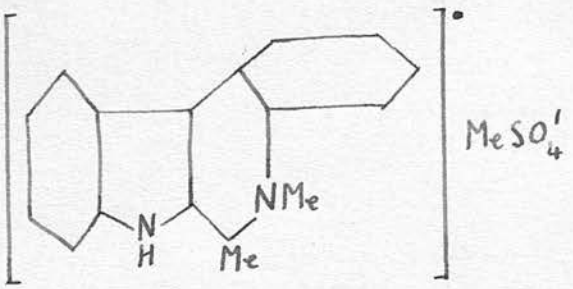
have the same properties in respect to forming metho-salts and anhydronium bases as the simple β-carboline derivatives. They are also capable of ready synthesis as described in the first section of this thesis, and they are therefore very suitable for the further investigation of the theory of anhydronium base formation. There seems little doubt that a rigorous proof of the constitution of the anhydronium bases and their metho-salts in this series would imply that a similar constitution holds in the β-carboline and other related series also.

Now according to the above theory, the anhydronium base formed from 4:5-benz-β-carboline methosulphate (LXI) should be a derivative of β-ψ-carboline (LVII) and should, therefore, possess the constitution LXII, and so the methosulphate of this anhydronium base ought to be identical with 1-methyl-4:5-benz-β-carboline methosulphate, which must have the constitution LXIII. This is so, as is shown below, and the identity of these two methosulphates, prepared quite independently, proves definitely that methyl sulphate adds itself on to the pyrrole nitrogen atom of the anhydronium base and, therefore, that the theory described above is correct.

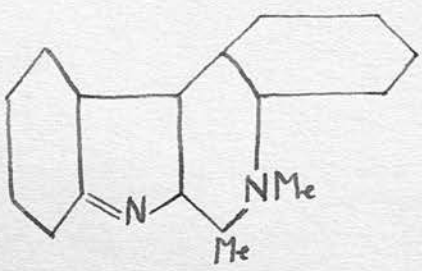
When/

When 4:5-benz-β-carboline (XXX), in benzene suspension, was heated with methyl sulphate, a bright yellow compound (m.p. 235°) was obtained, clearly 4:5-benz-β-carboline methosulphate (LXI). From an aqueous solution of this salt, an orange methohydroxide was readily precipitated by the addition of aqueous sodium hydroxide or ammonium hydroxide. When this methohydroxide was heated at 100° it lost a molecule of water with the formation of an anhydronium base (LXII), m.p. 205°, and this base readily reacted with methyl sulphate to give a methosulphate (LXIII), m.p. 300°. 1-Methyl-4:5-benz-β-carboline (XLV) reacted with methyl sulphate in benzene solution to yield a compound, m.p. 300°, which proved to be identical in all respects with the methosulphate of the anhydronium base which has been described above. This anhydronium base and its methosulphate are, therefore, properly named 3-methyl-4:5-benz-β-ψ-carboline and 1:3-dimethyl-4:5-benz-β-carbolinium methyl sulph-:ate respectively.

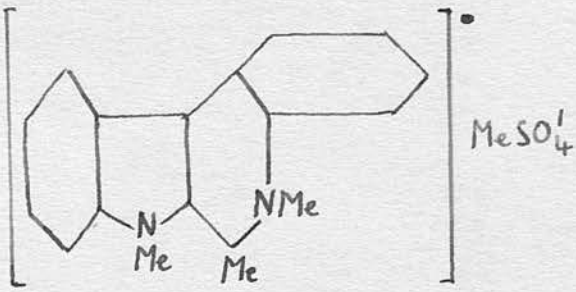
In order to confirm the conclusions just discussed, it was decided to repeat the work using the homologous series derived from 2-methyl-4:5-benz-β-carboline (XXXV) and 1:2-dimethyl-4:5-benz-β-carboline (XLVII)/



LXIV



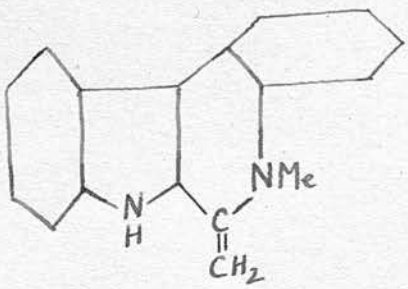
LXV



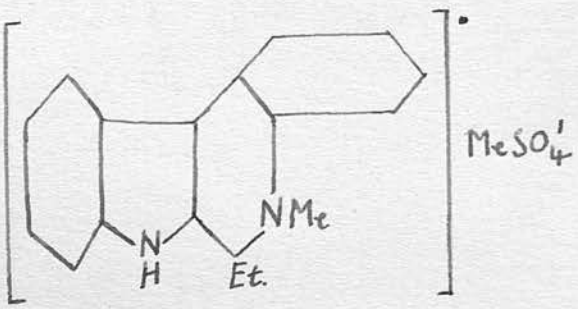
LXVI

(XLVII). 2-Methyl-4:5-benz-β-carboline methosulphate (LXIV), m.p. 270°, prepared in the usual way, yielded an anhydronium base (LXV), m.p. 225°, when treated with alkali, and this dry anhydronium base, dissolved in benzene, reacted readily with methyl sulphate with the formation of a methosulphate (LXVI), m.p. 292°. This compound proved to be identical in all respects with that formed when 1:2-dimethyl-4:5-benz-β-carboline was treated with methyl sulphate. Thus the above theory is once more confirmed. This anhydronium base and its methosulphate are, therefore, properly named 2:3-dimethyl-4:5-benz-β-ψ-carboline and 1:2:3-trimethyl-4:5-benz-β-carbolinium methyl sulphate respectively.

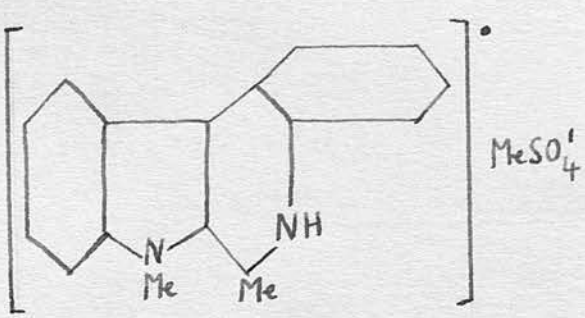
It may be mentioned that in this latter case considerable difficulty was encountered for when 1:2-dimethyl-4:5-benz-β-carboline and methyl sulphate were allowed to react together and the product recrystallised the compound obtained in the earlier experiments, although approximately pure, melted at 296° and when mixed with the compound, m.p. 292°, obtained from 2:3-dimethyl-4:5-benz-β-ψ-carboline, caused a depression of the melting point to about 272°. It was at first thought that the anhydronium base (LXV) might have reacted in the ethylenic form (LXVII) with methyl sulphate to form 2-ethyl-4:5-benz-β-carboline methosulphate (LXVIII). That this theory was,/



LXVII



LXVIII



LXIX

was, however, untenable was proved by forming the methosulphate of 2-ethyl-4:5-benz-β-carboline (XXXVII). This methosulphate (LXVIII), m.p. 250°, was clearly not identical with the product of m.p. 292°. The explanation of the difficulty was discovered when it was observed that the compounds which had a methyl group attached to the pyridine nitrogen atom gave no precipitate when excess of hydrochloric acid was added to a dilute aqueous solution, whereas compounds containing no methyl group in this position readily separated as sparingly soluble hydrochlorides. Now it was found that the compound of m.p. 296° gave a precipitate on the addition of hydrochloric acid and this precipitate proved to be identical with the hydrochloride of 1:2-dimethyl-4:5-benz-β-carboline. Further, when the compound of m.p. 296° was treated in aqueous solution with alkali, it was found that the resulting base, which could not readily be crystallised, on treatment with methyl sulphate in benzene solution, was converted into a methosulphate which melted at 292° and was identical in all respects with that obtained from 2:3-dimethyl-4:5-benz-β-ψ-carboline. The conclusion was drawn that the compound of m.p. 296° was a mixture of the desired compound (LXVI), m.p. 292°, and 1:2-dimethyl-4:5-benz-β-carbolinium methyl sulphate (LXIX), which presumably has/

has a higher melting point. The results of analysis, which as it happened agreed with those for the compound of m.p. 292°, also fitted in with this explanation. The formation of compound LXIX was apparently due to the presence of water or methyl hydrogen sulphate in the methyl sulphate used, and it was ultimately shown that all difficulties could be avoided if great care was taken to ensure that both the methyl sulphate and the benzene used were absolutely dry and neutral to litmus.

Since sodium hydroxide but not ammonium hydroxide precipitates the quaternary ammonium base from an aqueous solution of 1:3-dimethyl-4:5-benz-β-carbolinium methyl sulphate (LXIII), it is rather surprising to find that, in the case of 1:2:3-trimethyl-4:5-benz-β-carbolinium methyl sulphate (LXVI), the quaternary ammonium base is precipitated not only by sodium hydroxide but also by ammonium hydroxide (see pp.66-71). It would appear, therefore, that this latter base is somewhat weaker than the quaternary ammonium base of 1:3-dimethyl-4:5-benz-β-carbolinium methyl sulphate, which is precipitated by sodium hydroxide only.
In the case of methylharmine methosulphate (LVIII) Perkin and Robinson (loc. cit., p.949) found that even sodium hydroxide fails to precipitate the quaternary/

quaternary base in the cold, although it has been shown during the course of the present work that a very large excess of sodium hydroxide does effect precipitation. The extra benzene ring present in the 4:5-benz-β-carboline series therefore appears considerably to diminish the basic nature of the carboline nucleus.

III. Some Observations on the Fluorescence of 4:5-Benz-β-carboline and its Derivatives.

In view of the fact that a number of closely related fluorescent compounds were available, it appeared to be of some interest to investigate them systematically in order to ascertain whether any relation exists between the constitution of a compound and the colour of its fluorescence.

Solutions of the compounds were prepared by adding 0.1 cc. of an N/100-solution of the compound in water to 9.8 cc. of alcohol and 0.1 cc. of N-hydrochloric acid or of ammonium hydroxide (d 0.880). In this way acid and alkaline alcoholic solutions were obtained, each containing a compound at a concentration of N/10,000. These solutions, the fluorescence of which varied from blue to greenish-yellow, were arranged according to the colour of their fluorescences. In order to obtain reliable results they were arranged several times by independent observers, and after some practice the order could be determined with certainty.

It was found most convenient to illuminate the tubes by means of a carbon arc lamp as this produced a constant source of light and excited a very strong fluorescence/

TABLE I.

Compound.	Acid or alkali added.	Colour of fluorescence.	Highest dilution showing fluorescence.
1:2-Dimethyl-4:5-benz- β -carboline hydrochloride	NH_4OH	Blue	$N/10^6$
1-Methyl-4:5-benz- β -carboline hydrochloride	"	"	$N/2.10^6$
3-Methylharmine methosulphate	HCl	"	$N/10^7$
2-Ethyl-4:5-benz- β -carboline hydrochloride	NH_4OH	"	$N/4.10^5$
4:5-Benz- β -carboline hydrochloride	"	"	$N/10^6$
2-Methyl-4:5-benz- β -carboline hydrochloride	"	"	$N/4.10^5$
3-Methylharmine methosulphate	"	"	$N/10^7$
2-Ethyl-4:5-benz- β -carboline hydrochloride	HCl	Blue with greenish tinge	$N/2.10^7$
2-Methyl-4:5-benz- β -carboline hydrochloride	"	"	$N/10^7$
Quinine Hydrochloride	"	"	$N/10^7$
2:3-Dimethyl-4:5-benz- β - ψ -carboline hydrochloride	"	Greenish Blue	$N/2.10^7$
2-Methyl-4:5-benz- β -carboline methosulphate	"	"	$N/2.10^7$
2-Ethyl-4:5-benz- β -carboline methosulphate	"	"	$N/10^7$
1:2-Dimethyl-4:5-benz- β -carboline hydrochloride	"	Bluish green	$N/2.10^7$
4:5-Benz- β -carboline hydrochloride	"	"	$N/10^7$
2:3-Dimethyl-4:5-benz- β - ψ -carboline hydrochloride	NH_4OH	Green	$N/10^7$
2-Methyl-4:5-benz- β -carboline methosulphate	"	"	$N/10^7$
2-Ethyl-4:5-benz- β -carboline methosulphate	"	"	$N/10^7$
3-Methyl-4:5-benz- β - ψ -carboline hydrochloride	HCl	"	$N/2.10^7$
4:5-Benz- β -carboline methosulphate	"	"	$N/2.10^7$
1-Methyl-4:5-benz- β -carboline hydrochloride	"	"	$N/10^7$
1:2:3-Trimethyl-4:5-benz- β -carbolinium methyl sulphate	"	"	$N/10^7$
3-Methyl-4:5-benz- β - ψ -carboline hydrochloride	NH_4OH	Yellowish-green	$N/4.10^6$
4:5-Benz- β -carboline methosulphate	"	"	$N/4.10^6$
1:2:3-Trimethyl-4:5-benz- β -carbolinium methyl sulphate	"	"	$N/4.10^6$
1:3-Dimethyl-4:5-benz- β -carbolinium methyl sulphate	"	Greenish-yellow	$N/10^7$
1:3-Dimethyl-4:5-benz- β -carbolinium methyl sulphate	HCl	"	$N/10^7$
Quinine hydrochloride	NH_4OH	None at $N/10^4$	Less than $N/10^4$

fluorescence. The results are summarised in Table I. For the purposes of comparison 3-methylharmine methosulphate and quinine hydrochloride are included in this table. It may be noted here that N/10,000-solutions of these compounds in distilled water and in N/100-hydrochloric acid were also prepared but that the order of the colours of the fluorescences of these was identical with that of the hydrochloric acid-alcohol series. Addition of alkali to the aqueous solutions precipitated the bases in many cases and so destroyed the fluorescence. On this account the observations given in the table are those carried out on the alcoholic solutions. It may also be mentioned that alcoholic solutions containing sodium hydroxide (0.1 cc. of N/100-carboline solution, 9.7 cc. of alcohol, 0.2 cc. of 5N-sodium hydroxide) exhibited fluorescences identical with those of solutions containing ammonium hydroxide.

The chief deductions to be drawn from the table may be summarised as follows. A methyl (or ethyl) group attached to the carbon atom in position 2 (see formula XI) invariably renders the fluorescence more blue. With the introduction of a methyl group attached to either nitrogen atom, the fluorescence in acid solution becomes more yellow, the effect being greater when the methyl group is in position 1 than when it is in position 3. In acid solution the effect of a methyl group in position 2 is almost/

almost balanced by that of a methyl group in position 3. In alkaline solution a methyl group in the 3-position is much more powerful in making the fluorescence more green than one in the 2-position is in making it blue. It is of particular interest to note that 1-methyl-4:5-benz-β-carboline fluoresces bluer in alkaline solution than in acid solution, whereas 3-methyl-4:5-benz-β-ψ-carboline fluoresces yellower in alkaline solution than in acid solution. It seems a probable assumption that the yellowish-green fluorescent colour of 3-methyl-4:5-benz-β-ψ-carboline in alkaline solution is due to the existence of the ψ-carboline structure. In this connection it may be noted that the compounds, which contain a methyl group in the 3-position (that is, those compounds whose bases cannot possess a normal structure in alkaline solution), fluoresce more yellow in alkaline than in acid solution, whereas those compounds not possessing a methyl group in this position are bluer in alkaline than in acid solution. Hence it appears that derivatives of the normal base (4:5-benz-β-carboline) fluoresce very blue, particularly in alkaline solution, and incidentally that the bases 4:5-benz-β-carboline and 2-methyl-4:5-benz-β-carboline exist essentially in the normal form.

The figures given in the fourth column in Table I/

I represent the concentrations at which the fluorescences, when illuminated with a carbon arc lamp, are just visible. They are only approximate, but it is clear that the fluorescence of these compounds is usually considerably stronger in acid than in alkaline solution. Finally it may be observed that, in respect to colour and intensity of fluorescence, pairs of compounds such as 4:5-benz-β-carboline methosulphate and 3-methyl-4:5-benz-β-ψ-carboline hydrochloride, which contain the same organic kation, are identical.

EXPERIMENTAL.

Condensation of o-Nitrobenzyl Chloride and Ethyl Acetoacetate.

It has already been shown by Reissert (Ber., 1896, 29, 637) that, when o-nitrobenzyl chloride is condensed with ethyl acetoacetate in presence of sodium ethoxide, the product most readily obtained is derived from two molecules of o-nitrobenzyl chloride and one molecule of ethyl acetoacetate. Various attempts, therefore, were made to obtain the desired ethyl o-nitrobenzyl-acetoacetate by the use of a considerable excess of ethyl acetoacetate. Although this compound was not obtained, the following typical experiment indicates the method by which a new compound has been isolated in addition to that described by Reissert.

Ethyl acetoacetate (13 g.) was added to absolute ethyl alcohol (200 cc.) in which sodium (2.3 g.) had been dissolved. To this mixture, cooled to 0°, was added o-nitrobenzyl chloride (8.6 g.) dissolved in a minimum quantity of alcohol, and the whole was allowed gradually to attain room temperature; sodium chloride then slowly separated. After standing overnight, the mixture/

mixture was made slightly acid by the addition of hydrochloric acid (5%) and was then submitted to steam-distillation in order to remove the alcohol and any unchanged ethyl acetoacetate. The residue was exhaustively extracted with ether and the combined ethereal extracts were shaken with sodium hydroxide solution(1%). The ethereal solution gradually deposited crystals which, after recrystallisation from alcohol, had m.p. 103° and were found to possess the properties of the compound described by Reissert (loc. cit.). A current of carbon dioxide was bubbled through the combined alkaline extracts, and a brownish yellow crystalline precipitate slowly separated. This was filtered off and recrystallised several times from alcohol; it was then obtained as brownish yellow microscopic needles, m.p. 183° (decomp.) (Found: C, 60.0; H, 4.8; N, 6.6. $C_{20}H_{20}O_7N_2$ requires C, 60.0; H, 5.0; N, 7.0%). This new compound, for which formula XXIV appears to be the most probable, dissolves in sodium hydroxide solution but not in sodium carbonate solution. It is very insoluble in most of the usual organic solvents except boiling ethyl alcohol in which it dissolves to the extent of about two parts in a hundred. This low solubility in suitable solvents renders difficult the accurate determination of the molecular weight (Found: M,/

M, ebullioscopic in alcohol, 371. $C_{20}H_{20}O_7N_2$ requires M, 400). The compound dissolves in alcohol to give an intense reddish-brown solution.

o-Nitrophenylpyruvic Acid (XXVI).

Dry ether (500 cc.) was added to potassium (19.6 g.), previously powdered by shaking under hot toluene, and a mixture of absolute alcohol (24 g.) and ether (30 cc.) was introduced at such a rate that the ether did not boil too vigorously; after 6 hours the precipitation of the potassium ethoxide was usually complete. Freshly distilled ethyl oxalate (73 g.) was then added very slowly, care being taken to ensure that the temperature did not rise above 25°. To the clear orange solution so obtained, o-nitrotoluene (68.5 g., freshly distilled) was added and the mixture immediately became blood-red in colour. The neck of the flask was plugged with cotton wool and allowed to remain at 37° for 20 hours; a dark red crystalline mass, which consisted of the potassium derivative of o-nitrophenylpyruvic acid, then separated. The yellowish-brown supernatant ether layer was poured off and the solid washed twice by decantation with dry ether. It was then dissolved in water (400 cc.), and/

and this solution was extracted thrice with ether. When the solution had been freed from traces of ether by bubbling a current of air through it for 3 hours, it was acidified with a slight excess of hydrochloric acid. The o-nitrophenylpyruvic acid was precipitated as an orange-red mobile oil which solidified within a few minutes to a yellow crystalline cake. The weight of the dried acid was 84 g. A further quantity (12 g.) of less pure material was obtained from the ethereal washings of the potassium derivative and the ethereal extracts of the mother liquor from which the acid had been precipitated. Unless the above conditions are very carefully adhered to, the product obtained is of inferior quality and the yield poor. The acid crystallises from benzene in large, pale yellow, octagonal plates which melt at 121° (compare Reissert, Ber., 1897, 30, 1037; Blaikie and Perkin, J., 1924, 125, 332), but a much purer product m.p. 130°, and decomposing at 140° with evolution of gas, is obtained from alcohol.

o-Nitrophenylpyruvic Acid Phenylhydrazone (XXVII).

This compound was readily obtained when o-nitrophenylpyruvic acid (8.4 g.) was dissolved in acetic acid (50 cc. of 50%), an acetic acid solution of phenylhydrazine/

phenylhydrazine (4.8 g. in 20 cc. of 50% acetic acid) added, and the mixture heated for 30 minutes on the steam-bath. The viscid oil which separated solidified slowly on cooling, and crystallised from benzene in well-shaped pale yellow, prismatic needles, m.p. 153.5° with decomposition (Reissert, Ber., 1897, 30, 1038, gives m.p. 148-149°). The yield of the pure compound was 10.2 g.

o-Nitrophenylpyruvic acid phenylhydrazone is readily soluble in alcohol and glacial acetic acid, moderately easily soluble in ether and boiling benzene, and almost insoluble in cold benzene and light petroleum. It is only slightly soluble in cold dilute sodium hydroxide or sodium carbonate solution but dissolves slowly on warming, giving in the latter case a yellow solution from which the original material is deposited on cooling, and in the former case, giving ultimately a blood-red solution which smells of phenylhydrazine. Apparently in this case decomposition of the hydrazone takes place and the blood-red alkaline solution of o-nitrophenylpyruvic acid is obtained. The phenylhydrazone is insoluble in concentrated hydrochloric acid even on warming, and no indication of the formation, by this treatment, of any indole derivative could be obtained.

3-o-Nitrophenylindole-2-carboxylic Acid (XXVIII).

Although at first great difficulty was experienced in converting o-nitrophenylpyruvic acid phenylhydrazone into 3-o-nitrophenylindole-2-carboxylic acid by the Fischer method, it was ultimately found that this condensation was effected when an alcoholic solution of the phenylhydrazone was saturated with dry hydrogen chloride at the boiling point. As, however, it was found more convenient, in practice, to prepare the acid directly from o-nitrophenylpyruvic acid without isolation of the phenylhydrazone, the following procedure was adopted.

A mixture of o-nitrophenylpyruvic acid (80 g.), absolute ethyl alcohol (500 cc.), and phenylhydrazine (50 g.) was boiled for 30 minutes on the water-bath, cooled somewhat, and dry hydrogen chloride passed in; the solution then soon began to boil. During the process of saturation a brown solid made its appearance but this redissolved as saturation neared completion. When the alcohol was almost completely saturated with the gas, crystals of ammonium chloride separated, and the solution, previously a dark reddish-brown, became lighter in colour. When saturation was complete, the mixture was boiled for 30 minutes on the water-bath and again saturated with dry hydrogen chloride.

It/

It was then allowed to stand for a few hours, water (1000 cc.) added, and the oil which separated was extracted thrice with ether. After drying over anhydrous sodium sulphate and distilling off the ether, a dark red viscid oil was obtained. This oil could not be conveniently crystallised, but as a result of the insolubility of the potassium salt of the acid in alcohol the following method of purification and isolation of the acid was found very convenient. Alcoholic potassium hydroxide solution (30 g. of potassium hydroxide, 300 cc. of absolute alcohol), was added and the mixture boiled for 30 minutes on the water-bath. After standing overnight, the reddish-orange crystals which separated were collected, washed with absolute alcohol, and dried at 100° (yield, 55 g.) The free acid was liberated from this potassium salt as a pale yellow crystalline solid, which was dried and purified by recrystallisation from a mixture of equal parts of benzene and absolute alcohol. It was obtained in pale yellow, rectangular prismatic needles, m.p. 276° with decomposition and evolution of gas (Found: C, 64.1; H, 3.9; N, 9.8. $C_{15}H_{10}O_4N_2$ requires C, 63.8; H, 3.6; N, 9.9%).

3-o-Nitrophenylindole-2-carboxylic acid is insoluble in water, sparingly soluble in benzene and boiling chloroform, but it is much more readily soluble in alcohol. It dissolves readily in hot glacial acetic/

acetic acid from which it crystallises on cooling in pale greenish-yellow rhombic plates. The acid dissolves in cold dilute aqueous sodium hydroxide to give a yellow solution; the sodium salt is precipitated, however, when excess of sodium hydroxide solution is added. A similar behaviour is observed with ammonium hydroxide. The acid gives an orange coloration with p-dimethylaminobenzaldehyde and hydrochloric acid in alcoholic solution (Ehrlich's reagent) which is unchanged on prolonged boiling with excess of hydrochloric acid; no visible change is observed on the addition of aqueous sodium nitrite to the cooled solution.
No coloration is produced when the acid is treated with vanillin and hydrochloric acid even on prolonged boiling. The pine-shaving reaction is negative.

The following salts are precipitable from an aqueous solution of the ammonium salt of the acid: calcium salt, golden-yellow, crystalline, soluble in hot water, separates on cooling in sheaves of fine long prismatic needles; barium salt, pale yellow, crystalline, soluble in hot water, separates on cooling in large well-shaped rhombic plates; magnesium salt, bright yellow, crystalline, readily soluble in hot water, separates on cooling in feathery rosettes; lead and zinc salts, yellow, amorphous, insoluble in hot water.

The brucine salt separates readily when alcoholic solutions of the acid and of brucine are mixed. It is only slightly soluble in boiling alcohol and separates on cooling in fine, bright yellow, rectangular, prismatic needles, m.p. 230° (Found: N, 8.4. $C_{15}H_{10}O_4N_2$, $C_{23}H_{26}O_4N_2$ requires N, 8.3%. $[\alpha]_D^{16°}$ = -50.5° in 1% chloroform solution).

When the alcoholic mother liquors, from which potassium 3-o-nitrophenylindole-2-carboxylate had been crystallised, were diluted with a large excess of water an orange-red compound separated; this was filtered off and dried (39 g.) (The filtrate gave no precipitate on the addition of hydrochloric acid thus showing that the above method of separating 3-o-nitrophenylindole-2-carboxylic acid as its potassium salt was quantitative). Great difficulty was at first experienced in purifying this compound, but purification was ultimately effected as follows. The compound was dissolved in ether and the ethereal solution filtered from the insoluble tarry residue. The ether was then evaporated and the orange-red viscid oil which remained solidified readily on cooling. It crystallised from light petroleum (b.p. 60 - 80°), containing a little benzene, in long, bright orange prismatic needles which melted sharply at 119°. This compound proved to be 3-o-nitrophenylindole (XXXI) (Found:

C,/

C, 71.0; H, 4.6; N, 11.5. $C_{14}H_{10}O_2N_2$ requires C, 70.6; H, 4.2; N, 11.8%).

From a large number of experiments it has been found that the relative yields of 3-o-nitrophenylindole and of 3-o-nitrophenylindole-2-carboxylic acid are somewhat variable, and that when the mixture is kept boiling vigorously during the process of saturation with hydrogen chloride the yield of 3-o-nitrophenylindole tends to be large and that of 3-o-nitrophenylindole-2-carboxylic acid correspondingly small. An experiment was carried out in which the temperature during saturation was kept at 50°. The product was worked up as described above and an orange potassium salt was obtained which, however, tended to resinify on acidification of its solution in hot water and was ultimately shown to be the potassium salt of o-nitrophenylpyruvic acid phenylhydrazone.

2-Keto-2:3-dihydro-4:5-benz-β-carboline (XXIX).

Zinc dust (20 g.) was added gradually to a solution of 3-o-nitrophenylindole-2-carboxylic acid (6 g.) in boiling acetic acid (150 cc. of 80% acid), and the whole refluxed during 30 minutes. The mixture was then filtered and the residue extracted thrice with small/

small amounts of boiling acetic acid. The combined filtrates were poured into an excess of water and the pale pink solid which separated was collected and dried (4.5 g.). It was purified by recrystallisation from pyridine and separated in snow white, microscopic, rectangular, prismatic needles, which were unmolten at 316°. (Found: C, 76.4; H, 4.6. $C_{15}H_{10}ON_2$ requires C, 76.9; H, 4.3%).

In order to obtain information as to the ease with which the ring closed during the above preparation, an experiment was carried out in which the reduction was effected in alkaline solution with ferrous sulphate in presence of ammonia. When the mixture of 3-o-nitrophenylindole-2-carboxylic acid (2.8 g.) ammonium hydroxide (14 cc.; d 0.880), ferrous sulphate (18 g.) and water (40 cc.) was boiled for 2 hours oxidation of the ferrous oxide occurred. The filtrate from the mixture, however, gave no precipitate when acidified with hydrochloric acid, and it was therefore evident that the ammonium salt of 3-o-aminophenylindole-2-carboxylic acid was not in solution since in acid solution this would certainly lose water and the insoluble 2-keto-2:3-dihydro-4:5-benz-β-carboline would separate. The washed precipitate of iron oxide was extracted with dilute hydrochloric acid and the insoluble material (1.2 g.) which remained was recrystallised from pyridine, and

was found to be 2-keto-2:3-dihydro-4:5-benz- β - carboline. Thus it is clear that even in alkaline solution ring closure takes place, and this demonstrates the great readiness with which 3-o-aminophenylindole-2-carboxylic acid loses water.

2-Keto-2:3-dihydro-4:5-benz- β -carboline is practically insoluble in benzene, alcohol, and light petroleum even on boiling, but it is moderately easily soluble in hot glacial acetic acid and in pyridine. It is best recrystallised from the latter solvent since its solution in acetic acid tends to develop a pink coloration when exposed to the air and the crystals deposited from such a solution are also coloured. Its solution in acetic acid exhibits a fine blue fluorescence. A similar fluorescence is exhibited when the compound is boiled up with water containing a trace of hydrochloric acid. With Ehrlich's reagent this compound gives no reaction in the cold, but a very faint green coloration develops on prolonged boiling with excess of concentrated hydrochloric acid. This colour fades on cooling, and no further change is observed on the addition of aqueous sodium nitrite solution. A faint green coloration is also observed when this compound is treated with vanillin and hydrochloric acid. This colour does not change on prolonged boiling. The pine-shaving reaction is negative.

3-o-Nitrophenylindole (XXXI).

This compound, which was first obtained as described above as a by-product in the preparation of 3-o-nitrophenylindole-2-carboxylic acid, was also prepared by heating the latter in quantities of 2 g. at 275-280° when decomposition set in with the elimination of carbon dioxide. When the decomposition appeared to be complete the temperature was raised to 285° - 290°. The dark solid product, which was obtained on cooling, was boiled up several times with small quantities of aqueous sodium carbonate solution (5%) until all the unchanged acid was extracted. 3-o-Nitrophenylindole was isolated from the residual mass by means of ether and purified as described on p.40 . Owing to resinification at the high temperature, the yield was very poor. Various experiments were carried out with a view to increasing the yield of o-nitrophenylindole. The action of heat on the calcium or potassium salt of the acid, either at atmospheric pressure or in a vacuum, gave rise to a charred mass from which none of the desired product could be isolated. Similarly, no satisfactory results were obtained when the acid was heated in glycerol or quinoline solution. When, however, the ammonium salt of the acid was heated, in quantities of/

of 2 g., at 270 - 280° decomposition took place smoothly with evolution of carbon dioxide and ammonia. The product, on cooling, was dissolved in ether and the ethereal solution shaken up with small volumes of aqueous sodium carbonate solution (5%) until quite free from unchanged acid. The subsequent purification of the nitrophenylindole was effected as described above (yield, 87%).

3-o-Nitrophenylindole has a slight indole odour especially when heated, and is readily soluble in alcohol and benzene but much less soluble in light petroleum. It gives no colour reaction with Ehrlich's reagent in the cold, but on prolonged boiling with excess of concentrated hydrochloric acid a faint pink colour develops which fades on cooling. No change is observed on the addition of aqueous sodium nitrite to the cold solution. With vanillin and hydrochloric acid it gives no reaction in the cold but a pink coloration develops on warming. This colour disappears on dilution with water. The pine-shaving reaction is negative.

3-o-Aminophenylindole (XXXII).

Iron filings (20 g.) were added in small portions, to a constantly boiling solution of 3-o-nitrophenyl-indole/

3-o-nitrophenylindole (23.8 g.) in alcohol (200 cc. of 90% alcohol) containing concentrated hydrochloric acid (10 cc.; d 1.19), the complete addition occupying about 30 minutes. After all the iron had been added the mixture was boiled for 3 hours. Sodium ethoxide (4 g. of sodium in 100 cc. of alcohol) was then added and the whole refluxed for a few minutes. The mixture of iron and ferric hydroxide was filtered off and extracted thrice with small amounts of boiling alcohol. The combined filtrates were submitted to steam-distillation to remove the alcohol; the aminophenyl-indole then separated as a brown oil which solidified on cooling. The crude base (17 g.) was freed from any unchanged nitro compound by saturation of its ethereal solution with dry hydrogen chloride; the hydrochloride thus quantitatively precipitated was collected and washed with a little alcohol. The free base was liberated by warm aqueous sodium hydroxide solution and isolated by means of ether as a yellow oil which gradually solidified on cooling. Great difficulty was experienced in its further purification. It dissolved on warming in most organic solvents but separated on cooling as an oil which did not crystal-lise even when kept for a long time. When attempts were made to distil it under diminished pressure, it decomposed below the boiling-point, and pungent indole vapours were evolved. Ultimately it was found possible to/

to effect crystallisation by dissolving the crude base in boiling light petroleum (b.p. 80-100°) containing a little benzene, a few chips of potassium hydroxide being added to prevent the formation of the carbonate of the base. On cooling, the base separated partly as a yellow oil which solidified on standing, and partly in the form of fine long, colourless rectangular prismatic needles, m.p. 82°; on further recrystallisations the melting point fell to 75°. It would appear that the compound so obtained was not quite pure since analysis did not yield very satisfactory results (Found: C, 79.5; H, 6.1. $C_{14}H_{12}N_2$ requires C, 80.8; H, 5.8%).

3-o-Aminophenylindole has a slight aniline odour. It is readily soluble in alcohol and benzene but much less soluble in light petroleum. When hydrogen chloride is passed in to its alcoholic or ethereal solution, the hydrochloride readily separates in colourless, pear-shaped plates which melt at 288° with decomposition (Found: Cl, 14.5. $C_{14}H_{12}N_2$, HCl requires Cl, 14.5%). The hydrochloride is only sparingly soluble in water and the aqueous solution exhibits a very faint blue fluorescence. The picrate of the base separates in orange needles when solutions of the base and of picric acid in benzene are mixed. It recrystallises from benzene in stellate clusters of fine, long, orange, rectangular needles, m.p. 190°.

(Found/

(Found: N, 15.8. $C_{14}H_{12}N_2$, $C_6H_2(OH)(NO_2)_3$ requires N, 16.0%) Ehrlich's reagent gives with this indole derivative no reaction in the cold, but on prolonged boiling in presence of concentrated hydrochloric acid a faint pink coloration develops which disappears on cooling. The addition of aqueous sodium nitrite to the cooled solution effects no change. With vanillin and hydrochloric acid this base gives a faint pink coloration in the cold; this changes to green on warming but the colour fades on dilution. The pine-shaving reaction is negative. 3-o-Aminophenylindole hydrochloride is diazotised only very slowly, and the diazotised solution gives an orange-yellow precipitate with an alkaline solution of β-naphthol.

4:5-Benz-β-Carboline (XXX).

3-o-Aminophenylindole (2.3 g.) was formylated by boiling under reflux with an excess of formic acid (98-99%) for 15 minutes. When the reaction mixture was poured into excess of water, it was noticed that the latter exhibited a brilliant bluish-green fluorescence due to the formation of a trace of 4:5-benz-β-carboline by the dehydrating action of the formic acid (This small amount of carboline may be precipitated on making alkaline with excess of ammonium hydroxide/

hydroxide). The crude formyl derivative separated as an oil which solidified slowly, and when dried weighed 2 g.. It was dissolved in warm, perfectly dry toluene (8 cc.), freshly distilled phosphoryl chloride (5 cc.) was added, and the mixture was boiled under reflux in an oil-bath for 2 hours. During the earlier stages of the ebullition, hydrogen chloride was slowly evolved, and as the reaction neared completion a dark viscid oil separated, which solidified on cooling. After decantation of the supernatant toluene-phosphoryl chloride mixture, the solid was washed several times with light petroleum (b.p. 60-80°), dissolved in an excess of alcoholic potassium hydroxide (20%), and the solution filtered. The alkaline filtrate was then poured into an excess of water, and the resulting emulsion slowly deposited yellow, needle-shaped crystals of 4:5-benz- β -carboline. A preliminary purification was effected by dissolution of the crude carboline (1.4 g.) in boiling dilute hydrochloric acid, filtration, and precipitation from the filtrate with ammonium hydroxide. It was further purified by recrystallisation from benzene, containing a little solid potassium hydroxide, and separated in pale yellow, microscopic needles, m. p. 245°, (Found: C, 82.9; H, 4.8. $C_{15}H_{10}N_2$ requires C, 82.6; H, 4.6%) Acid solutions of 4:5-benz- β -carboline exhibit a beautiful bluish-green/

green fluorescence. The base gives none of the usual indole reactions.

4:5-Benz-β-carboline was also obtained from 2-keto-2:3-dihydro-4:5-benz-β-carboline (XXIX) as follows.

2-Keto-2:3-dihydro-4:5-benz-β-carboline (2.5 g.) was distilled over a large excess of zinc dust in a current of hydrogen. A small amount of a light brown oil condensed at a point in the unheated portions of the tube and solidified on standing. It was purified by recrystallisation from benzene, and the fine needle-shaped crystals which separated melted at 243°. This compound was shown to be identical with 4:5-benz-β-carboline since all its properties corresponded and the melting point of mixed specimens was 244°.

2-Methyl-4:5-benz-β-carboline (XXXV).

3-o-Aminophenylindole (16 g.) was dissolved in warm, freshly-distilled acetic anhydride (40 cc.) and the solution boiled under reflux for 30 minutes. When the reaction mixture was poured into excess of water, a fine blue fluorescence was observed (This fluorescence was due as in the case of the formyl derivative (vide supra) to the presence of a small amount of carboline (0.6 g.), formed during the acetylation/

acetylation). The aqueous mixture was warmed on the water-bath until decomposition of the unchanged acetic anhydride was complete. The oil which separated gradually solidified on cooling and was collected and dried (18.5 g.). The acetyl derivative crystallised from aqueous alcohol in colourless rhombic plates which, in a slightly impure condition, melted at about 158°.

3-o-Acetamidophenylindole is readily soluble in alcohol and benzene but only sparingly soluble in light petroleum.

Experiments were carried out with this acetyl derivative in order to ascertain whether condensing agents other than phosphoryl chloride would be effective in bringing about ring-closure. It was thought that saturation of the alcoholic solution of the acetyl derivative with dry hydrogen chloride would lead to the closing of the ring, but no carboline derivative could be detected by this means and the acetamido-:phenylindole was recovered unchanged. Even such powerful dehydrating agents as anhydrous zinc chloride and phosphorus pentoxide could effect no ring-closure when warmed with the acetyl derivative in alcohol and benzene solutions respectively. When, however, 3-o-acetamidophenylindole (7 g.) was dissolved in perfectly dry toluene (30 cc.), phosphoryl chloride (15 cc.) added, and the mixture boiled under reflux for/

for 2 hours, condensation took place as in the case of the formyl derivative (see p. 49). The carboline was isolated in the same way as in the case of the lower homologue. The crude base (6 g.) was purified by crystallisation from a mixture of benzene and light petroleum (b. p. 60 - 80°) containing chips of potassium hydroxide, and separated in stellate clusters of fine pale yellow, prismatic needles, m. p. 204-205° (Found: C, 83.1; H, 5.5; N, 12.0. $C_{16}H_{12}N_2$ requires C, 82.8; H, 5.2; N, 12.1%).

2-Methyl-4:5-benz- β -carboline is readily soluble in alcohol, benzene and chloroform, but much less soluble in light petroleum. Its solution in these solvents exhibits a brilliant blue fluorescence. The hydrochloride is precipitated in the form of fine bright yellow needles when hydrogen chloride is passed into an alcoholic solution of the base. The hydrochloride is only sparingly soluble in water and the saturated aqueous solution exhibits a vivid green fluorescence, which becomes blue on dilution, and is considerably reduced in intensity on the addition of sodium chloride solution. Aqueous sodium hydroxide, when added to the hydrochloride solution, precipitates the free base in the form of fine, pale yellow, microscopic needles. The addition of picric acid solution to the hydrochloride solution precipitates the picrate in bright yellow, hexagonal plates. In the same way chloroplatinic/

chloroplatinic acid solution precipitates the chloroplatinate in flat, sharp-pointed, yellow plates. The free base does not absorb carbon dioxide from the atmosphere and gives none of the usual indole reactions.

2-Ethyl-4:5-benz-β-carboline (XXXVII).

3-o-Aminophenylindole (20 g.) was boiled under reflux with an excess of propionic anhydride* for 30 minutes. The reaction product was poured into excess of water and the aqueous mixture warmed on the water-bath until decomposition of the unchanged propionic anhydride was complete. The crude propionyl derivative was dissolved in dry toluene (80 cc.) and refluxed for 2 hours with phosphoryl chloride (40 cc.). The product was worked up in the same way as in the case of the two lower homologues, and the crude base (14.5 g.) was recrystallised from benzene or ethyl acetate. 2-Ethyl-4:5-benz-β-carboline separated from these solvents in large, pale-yellow, rectangular plates (radially arranged), which melted at 158° with frothing. This frothing appears to be due to the loss of a small quantity of solvent of crystallisation at/

* Propionyl chloride in presence of excess of pyridine may be used to replace propionic anhydride.

at its melting point. It was found to be very difficult to remove all the solvent of crystallisation on prolonged heating at 100°, and the compound after this treatment still effervesced at its melting point and on analysis was found to contain C, 82.0; H, 6.1%. ($10C_{17}H_{14}N_2,C_4H_8O_2$ requires C, 82.0; H, 5.8%). This indicates that the last portion of ethyl acetate is not removed by prolonged heating at 100°.

2-Ethyl-4:5-benz-β-carboline has an intense bluish-green fluorescence in acid solutions. It does not give the ordinary indole reactions.

3-o-Nitrophenyl-1-methylindole-2-carboxylic Acid (XXXIX).

This acid was much more readily prepared than the corresponding 3-o-nitrophenylindole-2-carboxylic acid (XXVIII). A solution of o-nitrophenylpyruvic acid (42 g.) in warm glacial acetic acid (150 cc.) was diluted with an equal volume of boiling water. Phenylmethylhydrazine (25 g.) was then added and the mixture, after being warmed for a few minutes, was treated with hydrochloric acid (200 cc.; d 1.19), and was then heated on the water-bath for a further period of 30 minutes. The yellow oil which had separated readily solidified on cooling and, when filtered/

filtered off and dried, weighed 43 g.. It recrystallised from a small volume of hot alcohol in bright yellow, rectangular, prismatic needles, m. p. 234° (decomp.) (Found: C, 65.1; H, 3.9. $C_{16}H_{12}O_4N_2$ requires C, 64.9; H, 4.1%).

3-o-Nitrophenyl-1-methylindole-2-carboxylic acid is insoluble in water, sparingly soluble in benzene but is moderately easily soluble in alcohol. When it is boiled up with aqueous sodium hydroxide, a small amount is dissolved and the remainder is converted into a reddish-orange compound - apparently its sodium salt. A similar behaviour is observed in presence of ammonium hydroxide. This indole derivative gives no coloration with Ehrlich's reagent in the cold, but a faint pink coloration develops on prolonged boiling with excess of concentrated hydrochloric acid. This pink colour disappears on cooling, and no further change is observed on the addition of aqueous sodium nitrite solution. When the compound is warmed with vanillin and hydrochloric acid, a deep green coloration develops which persists on cooling but changes to yellow on dilution. No indole reaction could be observed with a pine-shaving moistened with hydrochloric acid and treated with a trace of the compound.

The following salts are precipitable from an aqueous solution of the ammonium salt of the acid: calcium salt, bright yellow, crystalline, soluble in/

in hot water, separates on cooling in fine prismatic needles which exhibit radial formation; barium salt, bright yellow, crystalline, soluble in hot water, separates on cooling in long slender prismatic needles; magnesium salt, orange-yellow, crystalline, moderately easily soluble in hot water, separates from a concentrated solution on cooling in well-shaped hexagonal plates; lead and zinc salts, bright yellow, amorphous, insoluble in hot water.

2-Keto-1-Methyl-2:3-Dihydro-4:5-Benz-β-Carboline (XL).

Small quantities of zinc dust were added to a boiling solution of 3-o-nitrophenyl-1-methylindole-2-carboxylic acid (2.5 g.) in acetic acid (100 cc.) until the yellow colour, which at first became a darker brown, gradually faded and practically disappeared. Boiling was continued under reflux for about 10 minutes and the mixture was then filtered. The zinc dust was repeatedly extracted with acetic acid until a test portion of the filtered extract, on dilution with water, showed practically no opalescence. The combined acetic acid filtrate and washings, which showed a marked fluorescence, were diluted with water (4 vols), and the pinkish precipitate was filtered off and/

and dried. It was recrystallised from pyridine, and was obtained in fine, white, felted needles, m. p. 302° (Found: N, 11.2. $C_{16}H_{12}ON_2$ requires N, 11.3%).

2-Keto-1-methyl-2:3-dihydro-4:5-benz- β -carboline is practically insoluble in water, alcohol, and benzene, but is slightly soluble in boiling glacial acetic acid from which it separates on cooling in small needles. It is more readily soluble in hot pyridine; the solution exhibits a very slight blue fluorescence. This fluorescence is more marked in acetic acid solution, and is also observed when a trace of the compound is boiled up with water containing a little hydrochloric acid. This carboline derivative gives none of the usual indole colour reactions.

3-o-Nitrophenyl-1-methylindole (XLI)

This compound was readily obtained when 3-o-nitrophenyl-1-methylindole-2-carboxylic acid was heated at 250° in a metal-bath. At this temperature decomposition took place smoothly with elimination of carbon dioxide. The reaction product, which melted under hot water, was repeatedly extracted with small amounts of boiling sodium carbonate solution (5%) until free from unchanged acid. After drying (yield, 80%/

80%), it was purified by recrystallisation from light petroleum (b.p. 80-100°) or alcohol. It separated from these solvents in large, orange octahedra, m.p. 98°. (Found: C, 71.4; H, 4.8; N, 11.2. $C_{15}H_{12}O_2N_2$ requires C, 71.4; H, 4.8; N, 11.1%).

3-o-Nitrophenyl-1-methylindole is readily soluble in benzene, chloroform, and glacial acetic acid, moderately easily soluble in alcohol, but only sparingly soluble in light petroleum. This indole derivative gives a faint pink coloration with Ehrlich's reagent in the cold. This colour is intensified slightly on prolonged boiling with hydrochloric acid, but it fades on cooling. The cooled solution gives an orange coloration with aqueous sodium nitrite solution. When the compound is treated with vanillin and hydrochloric acid, a pink colour develops in the cold and, on warming, this changes to a beautiful purple which persists on cooling but disappears on dilution. The pine-shaving reaction is negative.

3-o-Aminophenyl-1-methylindole (XLII and XLIII).

This compound was obtained from 3-o-nitrophenyl-1-methylindole by reduction with iron filings and hydrochloric acid under the same conditions as those used/

used to prepare 3-o-amino-phenylindole (XXXII). Iron filings (20 g.) were added in small quantities to a boiling solution of 3-o-nitrophenyl-1-methylindole (17 g.) in alcohol (400 cc.), containing hydrochloric acid (10 cc.). Boiling was continued for 3 hours and the reduction product was worked up as described in the case of 3-o-aminophenylindole except that the hydrochloride was obtained by passing dry hydrogen chloride into a solution of the crude base in benzene instead of in ether as the free base is rather insoluble in the latter solvent. The hydrochloride was reconverted to the free base by the action of excess of warm sodium hydroxide solution. The dried base (14.5 g.) was recrystallised from hot alcohol containing a little ammonium hydroxide, and separated on cooling in large, old-gold, rectangular prismatic needles, m.p. 129° (Found: C, 81.5; H, 6.3. $C_{15}H_{14}N_2$ requires C, 81.1; H, 6.3%).

3-o-Aminophenyl-1-methylindole is only slightly soluble in alcohol, light petroleum, and ether but it is readily soluble in benzene. Its sparing solubility in ether and alcohol is in contrast to the behaviour of 3-o-aminophenylindole itself. It is also curious in possessing a melting point 47° higher than that of the parent base, and in having a pronounced and distinctive colour. The hydrochloride, which melts at 246° with decomposition, separates in long, sharp pointed,/

pointed, colourless plates when a solution of the base in ether or in benzene is saturated with dry hydrogen chloride. The hydrochloride is only slightly soluble in water, ether, and benzene but it is readily soluble in alcohol. The picrate is precipitated when benzene solutions of the base and of picric acid are mixed. It separates from alcohol in bright orange, hexagonal prisms which melt at 196° and decompose at 205°. (Found: N, 15.3; $C_{15}H_{14}N_2,C_6H_2(OH)(NO_2)_3$ requires N, 15.5%).

3-o-Aminophenyl-1-methylindole gives a faint pink coloration with Ehrlich's reagent in the cold; this coloration is intensified slightly on boiling and fades on cooling. The colour of the cooled solution does not alter on the addition of aqueous sodium nitrite solution. With vanillin and hydrochloric acid, this indole base gives a pink coloration which is intensified on warming and fades on dilution. No colour reaction is observed when a pine-shaving moistened with hydrochloric acid is treated with a trace of this base. The hydrochloride of the base diazotises only very slowly and the diazotised solution gives an orange precipitate when treated with an alkaline solution of β-naphthol. No colour is developed when the free base is treated with sodium hypochlorite solution even on boiling.

1-Methyl-4:5-benz-β-carboline (XLV).

3-o-Aminophenyl-1-methylindole (5 g.) was formylated by boiling under reflux with an excess of formic acid (98-99%) for 15 minutes. When the reaction mixture was poured into excess of water, it was noticed that the latter exhibited a green fluorescence due to the formation of traces of 1-methyl-4:5-benz-β-carboline by the dehydrating action of the formic acid (The small amount of carboline formed may be precipitated by making alkaline with excess of ammonium hydroxide). The crude formyl derivative, which separated as a viscid oil, was extracted with ether and the ethereal solution dried over anhydrous sodium sulphate. After distillation of the ether, the residual oil was dissolved in warm, dry toluene (30 cc.). Phosphoryl chloride (12 cc.) was then added and the mixture boiled under reflux in an oil-bath for 2 hours. The supernatant toluene-phosphoryl chloride mixture was decanted from the solid, which had separated, and the latter washed thrice with light petroleum. It was then dissolved in an excess of alcoholic potassium hydroxide (20%) and the solution poured into ten times its volume of water. The carboline was precipitated as a crystalline mass and was purified by dissolution in boiling dilute hydrochloric acid and reprecipitation from the filtrate with ammonium hydroxide followed by recrystallisation/

recrystallisation from ligroin containing a little solid potassium hydroxide. It separated in long, pale pink, feathery needles, which exhibited radial formation, m.p. 142° (Found: C, 83.3; H, 5.4. $C_{16}H_{12}N_2$ requires C, 82.8; H, 5.2%).

1-Methyl-4:5-benz-β-carboline is readily soluble in alcohol, benzene and chloroform but much less soluble in ligroin and light petroleum. Its solution in these solvents exhibits a brilliant green fluorescence. The hydrochloride is only sparingly soluble in water and gives none of the usual indole reactions.

3-o-Acetamidophenyl-1-methylindole (XLVI).

3-o-Aminophenyl-1-methylindole (7.3 g.) was acetylated by boiling under reflux with an excess of acetic anhydride for 30 minutes. When the reaction mixture was poured into excess of water a fine green fluorescence was observed. This fluorescence was due, as in the case of the preparation of corresponding derivatives of the parent base, to the presence of a small amount of carboline formed during the acetylation. The aqueous mixture was warmed on the water-bath until decomposition of the unchanged acetic anhydride was complete. The crude acetyl derivative (7 g.), which solidified/

solidified on cooling, was purified by recrystallisation from alcohol, and separated in long pale-yellow, hexagonal prisms, m.p. 159°.

3-o-Acetamidophenyl-1-methylindole is only sparingly soluble in alcohol, ether and light petroleum but readily soluble in benzene, chloroform, and glacial acetic acid. No coloration is observed when a trace of this compound is treated with Ehrlich's reagent in the cold, but a pale green colour develops on prolonged boiling with excess of concentrated hydrochloric acid. This colour fades on cooling and the addition of aqueous sodium nitrite solution produces no further change. With vanillin and hydrochloric acid the acetyl derivative gives a faint purple coloration on warming which persists on cooling. The pine-shaving reaction is negative.

1:2-Dimethyl-4:5-benz-β-carboline (XLVII).

3-o-Acetamidophenyl-1-methylindole (4.5 g.) was dissolved in dry toluene (40 cc.) and refluxed for 2 hours with phosphoryl chloride (9 cc.). The carboline was worked up in the same way as in the case of the other homologues described above, and the crude base (2.9 g.) was purified by recrystallisation from hot alcohol/

alcohol containing a little ammonium hydroxide. It separated from this solvent in long pale yellow, rectangular plates, m.p. 154° (Found: C, 83.0; H, 5.7; $C_{17}H_{14}N_2$ requires C, 82.9; H, 5.7%).

1:2-Dimethyl-4:5-benz-β-carboline exhibits a beautiful green fluorescence in acid solutions, and gives none of the usual indole reactions.

4:5-Benz- β -Carboline Methosulphate (LXI).

Finely powdered 4:5-benz- β -carboline (3 g.) was warmed with a mixture of perfectly dry benzene (10 cc.) and methyl sulphate (2.5 g.) on the steam-bath. Combination took place readily and was complete in about 30 minutes. The greenish-yellow, crystalline product which was precipitated was filtered off, washed with a little benzene, and recrystallised from a small quantity of boiling methyl alcohol in which it was very soluble. The methosulphate separated in thick, bright yellow, prismatic needles, which exhibited a greenish fluorescence; m.p. 235°. (Found: C, 58.3; H, 5.3. $2C_{17}H_{16}O_4N_2S,CH_3OH$ requires C, 58.1; H, 5.0%).

4:5-Benz- β -carboline methosulphate is readily soluble in water and in methyl and ethyl alcohol. Its solution in these solvents exhibits a beautiful green fluorescence. Its solution in concentrated sulphuric acid also exhibits a green fluorescence which changes to deep greenish-blue on warming. The addition of sodium hydroxide or ammonium hydroxide to the aqueous solution gives at once a voluminous orange crystalline precipitate of the hydrated quaternary ammonium hydroxide.

3-Methyl-4:5-benz-β-ψ-carboline (LXII).

An aqueous solution of 4:5-benz-β-carboline methosulphate was treated with an excess of ammonium hydroxide in the cold. The orange hydrated quaternary ammonium hydroxide which separated was purified by recrystallisation from boiling water in which it was only sparingly soluble. The cooled solution slowly deposited fine, orange, prismatic needles which, after being heated at 100° for 6 hours, darkened at 190° and melted at 205°. (Found: C, 82.6; H, 5.2. $C_{16}H_{12}N_2$ requires C, 82.8; H, 5.2%).

3-Methyl-4:5-benz-β-ψ-carboline is readily soluble in alcohol and moderately easily soluble in benzene. Its solution in these solvents and in dilute hydrochloric acid exhibits a brilliant green fluorescence.

3-Methyl-4:5-benz-β-ψ-carboline Methosulphate (LXIII).

A benzene solution of 3-methyl-4:5-benz-β-ψ-carboline was boiled on the water-bath with a slight excess of methyl sulphate. The reaction was complete in about 30 minutes and the crude methosulphate which had precipitated was filtered off and purified by recrystallisation/

recrystallisation from boiling methyl alcohol. It separated in long, chrome-yellow, rectangular prismatic needles which exhibited a greenish fluorescence; m.p. 300°. (Found: C, 59.8; H, 5.2. $C_{18}H_{18}O_4N_2S$ requires C, 60.3; H, 5.0%).

3-Methyl-4:5-benz-β-ψ-carboline methosulphate is readily soluble in water and in boiling methyl alcohol. Its solution in these solvents exhibits a beautiful greenish-yellow fluorescence. Its solution in concentrated sulphuric acid also gives a greenish-yellow fluorescence but the colour changes to greenish-blue on warming. The addition of sodium hydroxide, but not ammonium hydroxide, to the aqueous solution precipitates the hydrated quaternary ammonium hydroxide as a voluminous, buff, amorphous compound.

1-Methyl-4:5-benz-β-carboline Methosulphate (LXIII).

1-Methyl-4:5-benz-β-carboline, in benzene solution, was warmed on the water-bath with a slight excess of methyl sulphate for 30 minutes. The methosulphate separated as a yellow crystalline mass and crystallised from methyl alcohol in long, chrome-yellow, rectangular, prismatic needles, which exhibited a greenish fluorescence; m.p. 300°.

1-Methyl-4:5-benz- β -carboline methosulphate was shown to be identical with 3-methyl-4:5-benz- β - ψ - carboline methosulphate (p.66) since all its properties corresponded and the melting-point of mixed specimens was 300°. This compound is, therefore, properly named 1:3-dimethyl-4:5-benz- β -carbolinium methyl sulphate.

2-Methyl-4:5-benz- β -carboline Methosulphate (LXIV).

2-Methyl-4:5-benz- β -carboline was allowed to react in benzene solution with a slight excess of methyl sulphate for 30 minutes on the steam-bath. The methosulphate was precipitated as a greenish-yellow, crystalline mass and was purified by re-crystallisation from boiling methyl alcohol. It separated in stout, bright yellow, prismatic needles, m.p. 270° (Found: C, 60.4, H, 5.2; N, 7.9. $C_{18}H_{18}O_4N_2S$ requires C, 60.3; H, 5.0; N, 7.8%).

2-Methyl-4:5-benz- β -carboline methosulphate is readily soluble in water and in boiling methyl alcohol. It separates from a hot concentrated aqueous solution on cooling in a voluminous mass of needles. Both the aqueous and alcoholic solutions exhibit a beautiful greenish-blue fluorescence.

Its/

Its solution in concentrated sulphuric acid exhibits a vivid greenish-blue fluorescence, which on warming changes to a brilliant blue and on dilution becomes bluish-green. The aqueous solution gives with sodium hydroxide or ammonium hydroxide a voluminous yellow crystalline precipitate of the hydrated quaternary ammonium hydroxide.

2:3-Dimethyl-4:5-benz-β-ψ-carboline (LXV).

To an aqueous solution of 2-methyl-4:5-benz-β-carboline methosulphate, ammonium hydroxide was added until precipitation of the hydrated quaternary ammonium hydroxide was complete. The base was purified by recrystallisation from boiling water. The cooled solution slowly deposited fine, long, yellow, felted needles which, after being heated for 6 hours at 100°, darkened slightly at 200° and melted at 225° (Found: C, 82.6; H, 5.8; N, 11.2. $C_{17}H_{14}N_2$ requires C, 82.9; H, 5.7; N, 11.4%).

2:3-Dimethyl-4:5-benz-β-ψ-carboline is readily soluble in alcohol and benzene. Its solution in these solvents and in dilute hydrochloric acid exhibits a brilliant greenish-blue fluorescence.

2:3-Dimethyl-4:5-benz- β - ψ -carboline Methosulphate (LXVI).

2:3-Dimethyl-4:5-benz- β - ψ -carboline was allowed to react in benzene solution with a slight excess of methyl sulphate on the steam-bath for 30 minutes. The crude methosulphate which separated crystallised from boiling methyl alcohol in long, chrome yellow, rectangular, prismatic needles which exhibited a bright green fluorescence m.p. 292° (Found: C, 61.2; H, 5.4; N, 7.3; $C_{19}H_{20}O_4N_2S$ requires C, 61.3; H, 5.4; N, 7.5%).

2:3-Dimethyl-4;5-benz- β - ψ -carboline methosulphate is readily soluble in water and in hot methyl alcohol. Its solution in these solvents exhibits a brilliant green fluorescence. It dissolves in concentrated sulphuric acid to give a vivid green fluorescent solution, but on warming the colour of the fluorescence changes to a brilliant blue and on dilution with water to greenish-blue. The addition of sodium hydroxide or ammonium hydroxide to the aqueous solution precipitates the quaternary ammonium hydroxide as a fine emulsion, from which yellow crystals slowly separate.

1:2-Dimethyl-4:5-benz- β -carboline Methosulphate (LXVI).

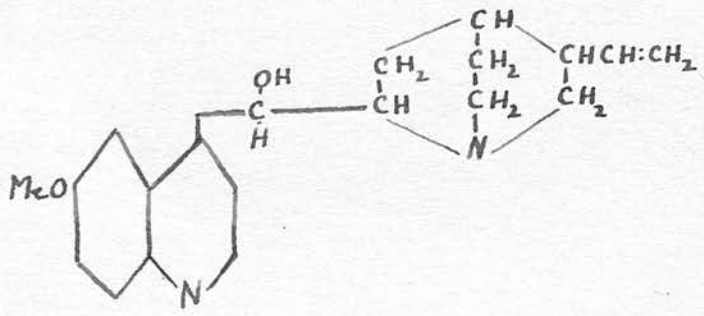
1:2-Dimethyl-4:5-benz- β -carboline readily reacted with methyl sulphate in boiling benzene solution. The resulting methosulphate was recrystallised from hot methyl alcohol, and separated in long, chrome-yellow, rectangular, prismatic needles which exhibited a bright green fluorescence, m.p. 292°.

1:2-Dimethyl-4:5-benz- β -carboline methosulphate was shown to be identical with 2:3-dimethyl-4:5-benz- β - ψ -carboline methosulphate (p.70) since all its properties corresponded and the melting-point of mixed specimens was 292°. This compound is, therefore, properly named 1:2:3-trimethyl-4:5-benz- β -carbolinium methyl sulphate.

2-Ethyl-4:5-benz- β -carboline Methosulphate (LXVIII).

A benzene solution of 2-ethyl-4:5-benz- β -carboline was boiled on the water-bath with a slight excess of methyl sulphate for 30 minutes. The crude methosulphate which separated crystallised from boiling methyl alcohol in pale yellow, tetragonal plates, m.p. 250°. (Found: C, 61.6; H, 5.6; $C_{19}H_{20}O_4N_2S$ requires C, 61.3; H, 5.4%).

2-Ethyl-4:5-benz- β -carboline methosulphate is readily soluble in water and in boiling methyl alcohol. Its solution in these solvents exhibits a greenish-blue fluorescence. Its solution in concentrated sulphuric acid also gives a greenish-blue fluorescence but this changes on warming to brilliant blue. The addition of sodium hydroxide or ammonium hydroxide to the aqueous solution gives at once a voluminous, bright yellow, crystalline precipitate of the hydrated quaternary ammonium hydroxide.


MeO
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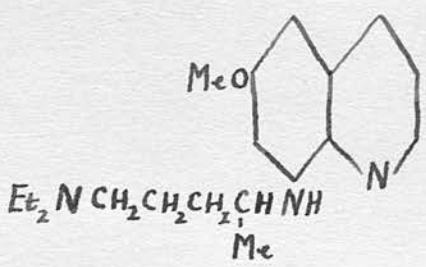
CH
CHCH:CH2
CH2
N

I


MeO
N
Et2N CH2CH2CH2CH NH
Me

II.

PART II.

QUINOLINE COMPOUNDS CONTAINING ARSENIC.

INTRODUCTION.

Until quite recently the compound which has been most successful in combating malaria is quinine (I) - an alkaloid obtained from Cinchona bark. Whilst quinine is quite efficacious in the treatment of certain forms of malaria, it has several disadvantages the most important of which is that it appears to have comparatively little if any influence on the sexual form (gametocyte) of the parasite responsible for the disease. A considerable amount of research work has been carried out during the past few years with a view to preparing compounds which are therapeutically active in malaria, and as a result the synthetic compound known as plasmoquine or beprochin (Bayer) (II) has been put on the market (compare Barger and Robinson, J., 1929, 2947). This quinoline derivative possesses definite anti-malarial properties and unlike quinine attacks mainly the sexual form of the parasite. A mixture of quinine and plasmoquine has proved to be of especial value in the treatment of malaria in particular cases, but the toxic effects of plasmoquine constitute/

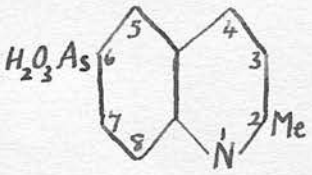
constitute a certain disadvantage, and there seems every reason to believe that further research would result in the preparation of a compound giving even better clinical results.

Since quinine (I) and plasmoquine (II) both contain a 6-methoxyquinoline nucleus, it would be expected on a priori grounds that the existence of such a quinoline ring in a compound would enhance its therapeutic value. As mentioned above, in certain types of malaria (particularly in the chronic form) quinine has only a slight curative action, but it has been claimed that such cases frequently respond favourably to arsenic treatment. It is of course well known that organic compounds of arsenic possess great therapeutic activity in certain spirochaetal and trypanosomal diseases and it appears therefore highly probable that an arsenic derivative of quinoline might be found which would possess anti-malarial activity to a marked degree. It was therefore considered desirable to synthesise quinoline compounds containing arsenic in order that their therapeutic activities might be ascertained. The present investigation describes methods for the synthesis of such compounds. This thesis is concerned only with the chemical aspects of the work and the chemotherapeutic tests are not dealt with.

From/

From a study of the literature it appears that the first arsenic derivative of quinoline was made by Schiff (Annalen, 1864, 131, 116) who found that quinoline readily reacted with arsenious chloride with the formation of an addition compound possessing the empirical formula $C_9H_7N,AsCl_3$. Fränkel and Löwy (Ber., 1913, 46, 2546) confirmed this observation and also obtained in a similar manner addition compounds of 8-hydroxy-quinoline and tetrahydroquinoline with arsenious chloride. Fränkel and Löwy (loc. cit.) also claimed to have obtained a 2-methylquinolylarsonic acid ($C_{10}H_{10}O_3NAs$) by applying the Döbner-Miller reaction to arsanilic acid (p-aminophenylarsonic acid) and acetaldehyde. These investigators appeared to be uncertain as to which carbon atom the arsonic acid group is attached in this 2-methylquinolylarsonic acid, but if they started with pure p-aminophenylarsonic acid then it would be expected that the compound in question would be 2-methylquinoline-6-arsonic acid (III). Since, however, they state that their acid was insoluble in mineral acids and in sodium hydroxide solution, it seems probable in the light of our present knowledge of the properties of quinolylarsonic acids that their compound did not possess this constitution.

Since this time comparatively little work has been carried out on the synthesis of quinoline compounds/



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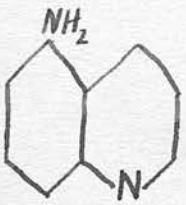

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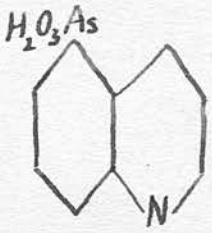

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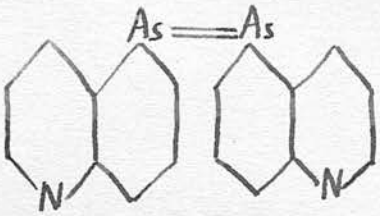

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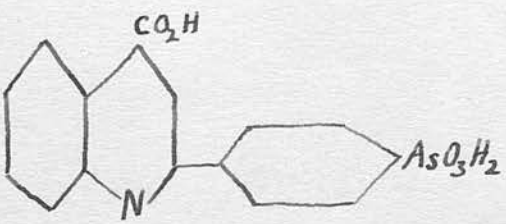
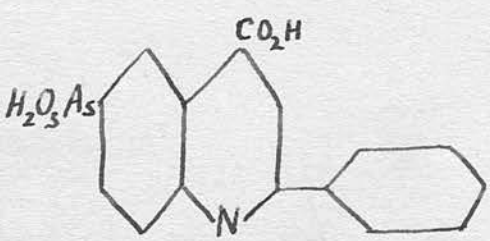


VII

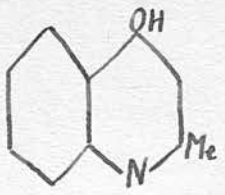


VIII.

compounds containing arsenic and those synthesised may be divided into the following four groups:-

(a) Simple quinoline compounds containing an arsonic acid group directly attached to the quinoline nucleus and certain of their arseno-derivatives. (Binz and Räth, Annalen, 1927, 453, 238; Fourneau, Tréfouel and Benoit, Ann. Inst. Pasteur, 1930, 44, 719; Balaban, J., 1930, 2346). These quinolylarsonic acids were all obtained from the corresponding amino-compounds by means of the Bart reaction. Thus, for example, 5-aminoquinoline (IV) was converted through its diazonium chloride into quinoline-5-arsonic acid (V), which on reduction with sodium hypophosphite gave 5-5'-arsenoquinoline (VI) (Binz and Räth, loc. cit.).

(b) 2-Phenylquinoline-4-carboxylic acid("atophan") containing an arsonic acid group directly attached either to the benzene nucleus or to the quinoline nucleus. Thus p-4'-carboxy-2'-quinolylphenylarsonic acid (VII) was prepared by condensation of acetophenone--p-arsonic acid with isatin, whilst 2-phenyl-4-carboxy-quinoline-6-arsonic acid (VIII) was obtained from the corresponding amino-compound by the Bart reaction (Ogden and Adams, J. Amer. Chem. Soc., 1925, 47, 827; Calvery, Noller and Adams, ibid., 1925, 47, 3058). These arsonic acids were converted into the corresponding arseno-derivatives by reduction with sodium hydrosulphite.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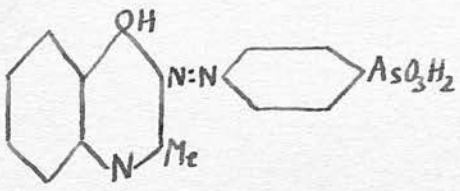
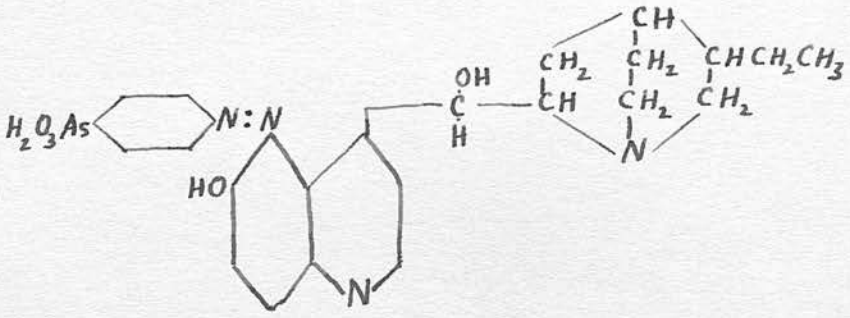
Me
N

IX.


OH
N:N
AsO3H2
Me
N

X.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HO
OH
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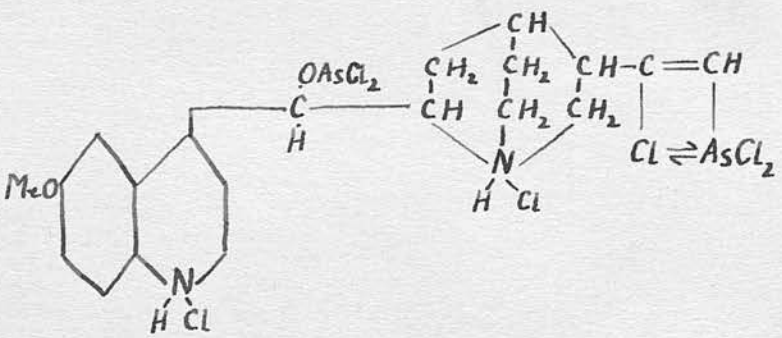
CH2
CH2
CHCH2CH3
CH
CH2
CH2
CH2
N
N

XI.


MeO
OAsCl2
CH
CH2
CH2
CH-C=CH
CH
CH2
CH2
Cl⇌AsCl2
N
H Cl
N
H Cl

XII.

(c) Quinolylazophenylarsonic acids. These arsonic acids were prepared by coupling hydroxy- or amino-quinolines with diazotised p-aminophenylarsonic acid. Thus, for example, p-4'-hydroxy-2'-methyl-3'--quinolylazophenylarsonic acid (X) was obtained by coupling 4-hydroxy-2-methylquinoline (IX) with the diazo-chloride obtained from p-aminophenylarsonic acid (Berlingozzi, Annali Chim. Appl., 1928, 18, 31, 333). To this group also belongs dihydrocupreine-5-azobenzene--p-arsonic acid (XI) obtained from dihydrocupreine and diazotised p-aminophenylarsonic acid (Erben and Schniderschitsch, Ber., 1925, 58, 693).

(d) Arsenic derivatives of quinine, dehydroquinine and dihydroquinine. These compounds were all prepared by treating the above bases with arsenious chloride. Thus, for example, dehydroquinine and arsenious chloride react together to yield a compound to which the constitution XII has been assigned (compare Erben, Philippi and Schniderschitsch, Ber., 1925, 58, 2854; Erben and Philippi, ibid., 1927, 60, 122; Erben, ibid., 1928, 61, 2106).

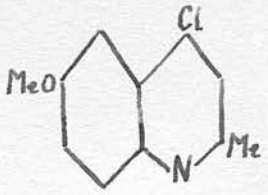
The present investigation on the synthesis of quinoline compounds containing arsenic is divided into the following three sections:-

I. Synthesis of quinoline derivatives of amino-phenylarsonic acids.

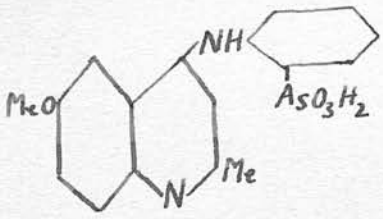
II./

II. Synthesis of quinbenzarsazine derivatives.

III. Synthesis of quinoline derivatives containing an arsonic acid group directly attached to the quinoline nucleus. This section constitutes an investigation into the methods available for the synthesis of new compounds belonging to group (a) (above) and opens up a way for the synthesis of quinoline analogues of stovarsol and salvarsan.



XIII.



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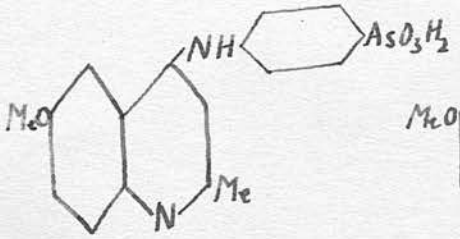

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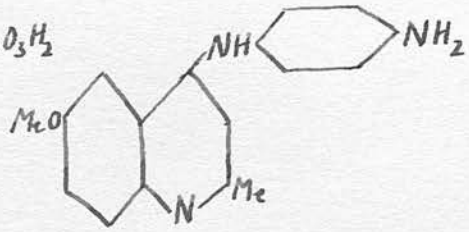
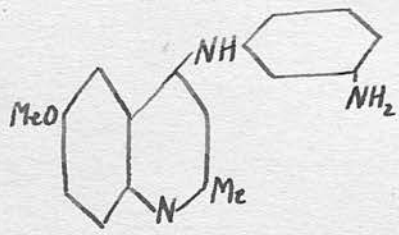
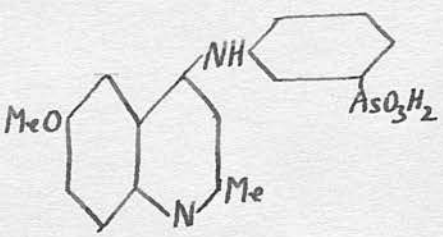


XVI.



XVII.



XVIII.

I. SYNTHESIS OF QUINOLINE DERIVATIVES OF AMINOPHENYLARSONIC ACIDS.

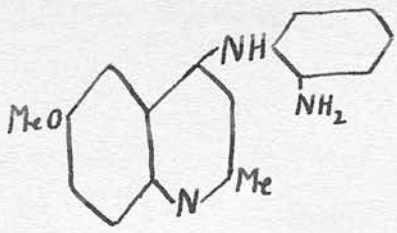
The comparative ease of preparation of 4-chloro--2-methylquinoline compounds from aniline derivatives and the labile nature of the halogen atom in such compounds makes them particularly suitable as initial-materials for the synthesis of quinoline derivatives of aminophenylarsonic acids. The first method of synthesis investigated was that of direct condensation of an aminophenylarsonic acid with 4-chloro-6-methoxy--2-methylquinoline (XIII), which was prepared from p-anisidine and ethyl acetoacetate as described by Conrad and Limpach (Ber., 1888, 21, 1651). Although a considerable number of experiments were carried out with both o-aminophenylarsonic acid and p-aminophenyl--arsonic acid, it was not found possible to isolate the desired quinolylaminophenylarsonic acids (XIV and XV). These experiments included heating the chloro-methylquinoline with the aminophenylarsonic acid (a) in the dry state, (b) in presence of quinoline, and (c) in boiling amyl alcohol to which anhydrous potas-sium carbonate and traces of copper powder and cuprous iodide had been added (compare Burton and Gibson, J., 1926, 459; Wintersteiner and Lieb, Ber., 1928, 61, 1126). In all cases the original compounds could be/

be recovered unchanged except where extensive decomposition had set in. The synthesis of quinolylamino--phenylarsonic acids was however successfully accomplished by a method which is described below.

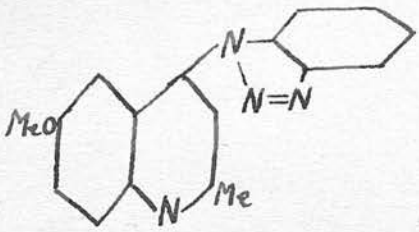
When 4-chloro-6-methoxy-2-methylquinoline was heated with an excess of p-phenylenediamine under diminished pressure at 130-140°, 4-p-aminoanilino-6--methoxy-2-methylquinoline (XVI) was readily produced. This base was converted into p-6'-methoxy-2'-methyl--4'-quinolylaminophenylarsonic acid (XV) by means of the Bart reaction. It was found that when diazotisation was carried out in aqueous mixture the yields of arsonic acid obtained were somewhat erratic. Diazotisation of the base, however, in glacial acetic acid and treatment of the solid diazo-compound so obtained with sodium arsenite gave a uniform yield of quinolylaminophenylarsonic acid.

By the substitution of m-phenylenediamine for p-phenylenediamine in the above series of reactions, it was possible to prepare in a similar manner 4-m-aminoanilino-6-methoxy-2-methylquinoline (XVII) and m-6'-methoxy-2'-methyl-4'-quinolylaminophenyl--arsonic acid (XVIII).

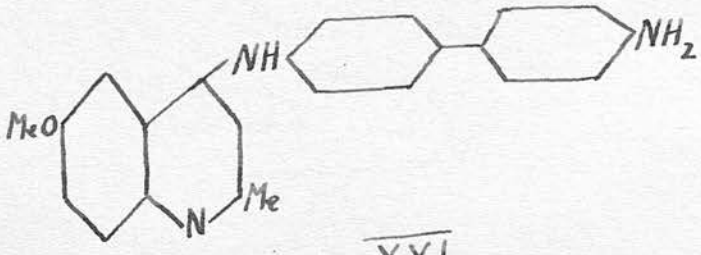
It was not found possible to prepare o-6'-methoxy--2'-methyl-4'-quinolylaminophenylarsonic acid (XIV) from 4-o-aminoanilino-6-methoxy-2-methylquinoline (XIX)/



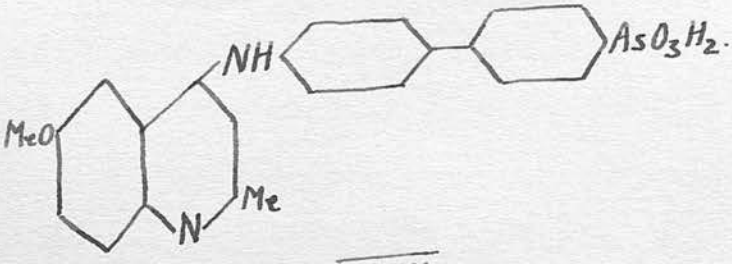
XIX



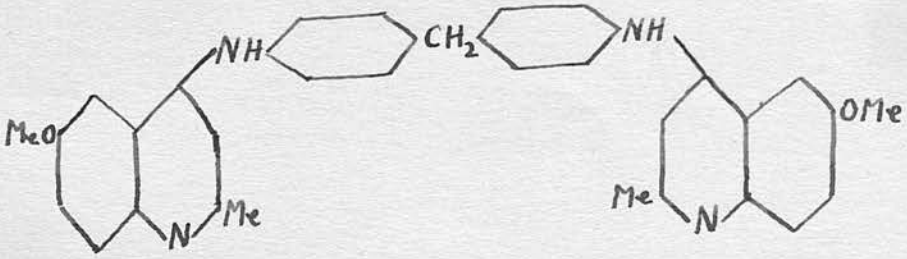
XX



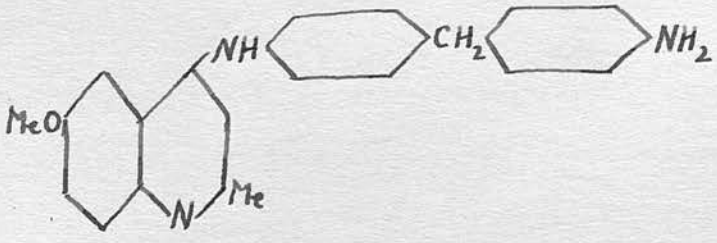
XXI.



XXII



XXIII



XXIV.

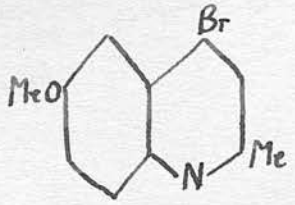
(XIX) in the same way since this latter compound, on treatment with nitrous acid, is readily converted into 4-(benztriazolyl-3')-6-methoxy-2-methylquinoline (XX) (compare Kermack and Smith, J., 1930, 2004).

4-Chloro-6-methoxy-2-methylquinoline and benzidine condensed readily when heated together at 160° in presence of excess of the latter with formation of 4-benzidino-6-methoxy-2-methylquinoline (XXI). This base was converted into 4'-6"-methoxy-2"-methyl--4"-quinolylaminodiphenylarsonic acid (XXII) by means of the Bart reaction.

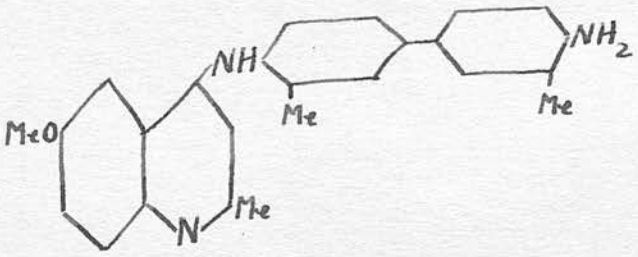
o-Tolidine and o-dianisidine reacted only very slowly when heated with 4-chloro-6-methoxy-2-methyl--quinoline, and mono-quinolyl derivatives of these two diphenyl bases could not be obtained in this way.

pp'-Diaminodiphenylmethane and 4-chloro-6-methoxy--2-methylquinoline condensed slowly when heated together at 130° with production of pp'-di-6-methoxy-2-methyl-4-quinolyldiaminodiphenylmethane (XXIII). All attempts to prepare p-6-methoxy-2-methyl-4--quinolylamino-p'-aminodiphenylmethane (XXIV) even by the use of a large excess of pp'-diaminodiphenyl-methane completely failed, the sole condensation product isolated being the di-substituted compound (XXIII).

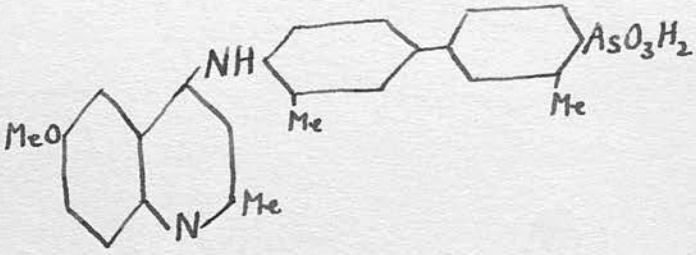
Since it is well known that derivatives of bromobenzene/



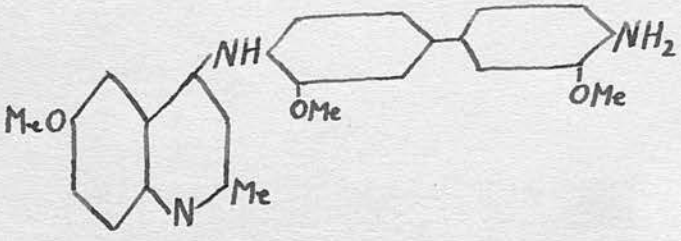
XXV



XXVI



XXVII.



XXVIII.

bromobenzene are more reactive than the corresponding chloro-compounds, it appeared desirable to ascertain whether the required products could be obtained by condensation of o-aminophenylarsonic acid, o-tolidine, o-dianisidine and pp'-diaminodiphenylmethane with 4-bromo-6-methoxy-2-methylquinoline instead of with 4-chloro-6-methoxy-2-methylquinoline. This modification proved entirely successful.

4-Bromo-6-methoxy-2-methylquinoline (XXV) was prepared from 4-hydroxy-6-methoxy-2-methylquinoline (Conrad and Limpach, loc. cit. p.1650) by the action of phosphoryl bromide provided that the temperature of the reaction was carefully controlled.

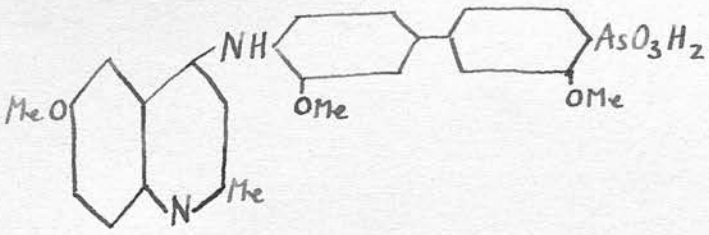
When o-aminophenylarsonic acid was boiled in amyl alcoholic solution with 4-bromo-6-methoxy-2-methyl--quinoline to which anhydrous potassium carbonate and traces of finely divided copper-bronze and iodine had been added, condensation took place slowly with formation of o-6'-methoxy-2'-methyl-4'-quinolylaminophenylarsonic acid (XIV). This arsonic acid closely resembles its m- and p- isomerides (XVIII and XV) and possesses amphoteric properties.

o-Tolidine and 4-bromo-6-methoxy-2-methylquinoline condensed slowly when heated together at 140-150° in presence of considerable excess of the former with production of 4-o-tolidino-6-methoxy-2-methylquinoline (XXVI/

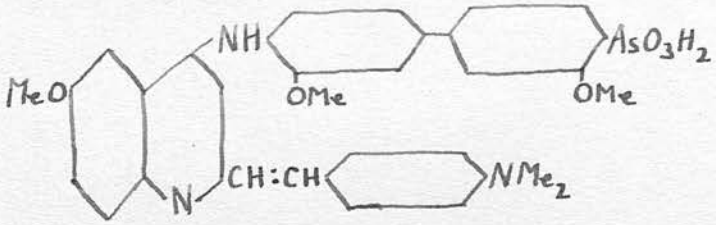
(XXVI). This compound was readily converted into 4'-6"-methoxy-2"-methyl-4"-quinolylamino-3:3'-dimethyl--diphenylylarsonic acid (XXVII) by means of the Bart reaction.

Condensation of o-dianisidine with 4-bromo-6--methoxy-2-methylquinoline proceeded very slowly at 135-140° accompanied by a certain amount of decomposition, and only a small quantity of 4-o-dianisidino-6--methoxy-2-methylquinoline (XXVIII) could be isolated from the reaction-mixture. However, by the use of a partial vacuum (10-15 mm. pressure) the reaction took place rapidly without decomposition setting in, and a very good yield of the above mono-quinolyldianisidine compound was obtained. To confirm this observation two experiments were carried out side by side in the same oil-bath one being open to the air and the other connected to the water-pump through a water-trap. Slight frothing appeared in the experiment carried out in vacuo, and the mixture became very viscous in a few minutes. No gas, however, was evolved as there was no bubbling through the water-trap nor did any acid accumulate there; an excellent yield of condensation product was obtained. In the experiment carried out at atmospheric pressure practically no reaction appeared to take place, and only a slight increase in viscosity set in; a very small amount of condensation-product

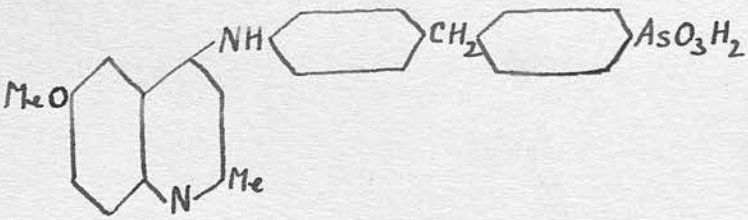
was/



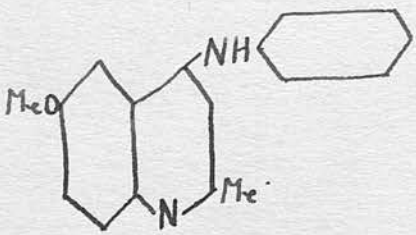
XXIX



XXX



XXXI



XXXII

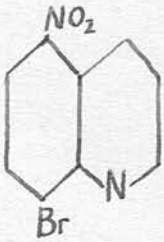
was obtained from the resinous reaction-mixture. The effect of the vacuum is probably to remove the last traces of water-vapour which would appear to interfere with the reaction. The long time required in the absence of a vacuum results in the occurrence of various side reactions including demethylation and the consequence of formation of resinous by-products partially soluble in sodium hydroxide solution.

4-o-Dianisidino-6-methoxy-2-methylquinoline (XXVIII) was readily converted into 4'-6"-methoxy--2"-methyl-4"-quinolylamino-3-3'-dimethoxydiphenylyl-arsonic acid (XXIX) by means of the Bart reaction. The yield of purified product was very good (71% of the theoretical). Attempts were made to condense this arsonic acid with p-dimethylaminobenzaldehyde and in this way introduce a styryl group into the molecule. However, when a mixture of the arsonic acid (XXIX) and p-dimethylaminobenzaldehyde in alcoholic sodium hydroxide solution was boiled under reflux for 6 hours in presence of piperidine, no trace of compound XXX could be detected and the original acid was recovered unchanged. Since the methyl group in the simple 2-methylquinoline derivatives readily reacts with p-dimethylaminobenzaldehyde under the above conditions (compare Browning, Cohen, Ellingworth and Gulbransen, Proc. Roy. Soc., London, 1926, 100, 293), it appears that/

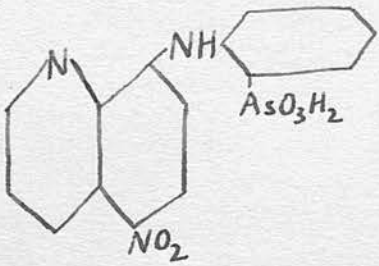
that the reactivity of the similarly situated methyl group in compound XXIX is considerably diminished in presence of the diphenylylarsonic acid group.

pp'-Diaminodiphenylmethane and 4-bromo-6-methoxy--2-methylquinoline condensed readily when heated together, under diminished pressure (10-15 mm.), at 130° in presence of considerable excess of the former. The product of the reaction was found to be p-6-methoxy--2-methyl-4-quinolylamino-p'-aminodiphenylmethane (XXIV); no trace of the corresponding diquinolyl derivative (XXIII), which was obtained from 4-chloro--6-methoxy-2-methylquinoline and pp'-diaminodiphenyl--methane, could be isolated. It is very difficult to suggest a reason as to why the diquinolyl derivative is the sole product of the reaction between pp'-diamino--diphenylmethane and 4-chloro-6-methoxy-2-methylquinoline whilst only the monoquinolyl derivative is formed by the use of 4-bromo-6-methoxy-2-methylquinoline instead of the corresponding chloro-compound. Compound XXIV was converted into p-6-methoxy-2-methyl--4-quinolylaminodiphenylmethane-p'-arsonic acid (XXXI) in the usual way.

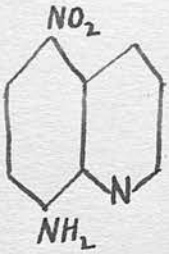
The parent base, namely 4-anilino-6-methoxy-2--methylquinoline (XXXII), of which the above compounds are derivatives has not been previously described. It was readily obtained by condensation of aniline with 4-chloro-6-methoxy-2-methylquinoline at 180°.



XXXIII



XXXIV



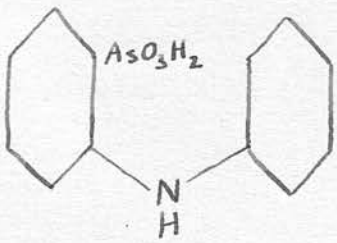
XXXV

In order to obtain a quinolylaminophenylarsonic acid in which the aminophenylarsonic acid group is attached to the quinoline nucleus in the 8-position instead of in the 4-position, attempts were made to condense o-aminophenylarsonic acid with 8-bromo-5-nitroquinoline (XXXIII). This latter compound was prepared from 8-aminoquinoline by a modification of the method of Dikshoorn (Rec. trav. chim., 1929, 48, 550). These condensation experiments were however unsuccessful, whether the two substances were heated together (a) in boiling amyl alcohol in presence of anhydrous potassium carbonate with or without the addition of traces of finely divided copper-bronze and iodine, (b) in the dry state, or (c) in presence of pyridine. The main product of the reaction from the experiment carried out under (a) proved to be 5-nitro-8-hydroxyquinoline and only a small amount of what was probably impure o-5'-nitro-8'-quinolylamino-phenylarsonic acid (XXXIV) was isolated. In the other cases the original compounds could be recovered unchanged except where extensive decomposition had set in on account of the prolonged heating at a high temperature. Compound XXXIV was, however, obtained in a pure condition but in somewhat poor yield by condensation of o-bromophenylarsonic acid with 5-nitro--8-aminoquinoline (XXXV) in presence of amyl alcohol.

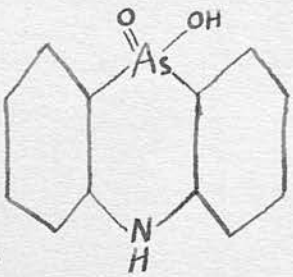
The/

The 5-nitro-8-aminoquinoline used in this reaction was prepared in quantitative yield from 8-bromo-5-nitroquinoline by treatment with methyl alcoholic ammonia at 140° for 4 hours. This is a very convenient method for the preparation of this otherwise rather inaccessible base (compare Dikshoorn, Rec. trav. chim, 1929, 48, 517).

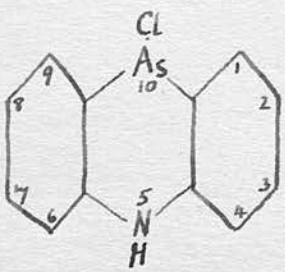
Most of the new compounds described above give brilliant colorations ranging from red to purple when they are added under suitable conditions to a dilute solution of iodine in aqueous potassium iodide. These reactions are fully described in the Proceedings of the Royal Society, Edinburgh (1930, 50, 243); a reprint of the paper is enclosed at the back of this thesis.



XXXVI



XXXVII



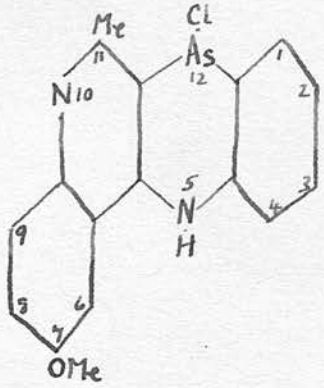
XXXVIII

II. SYNTHESIS OF QUINBENZARSAZINE DERIVATIVES.

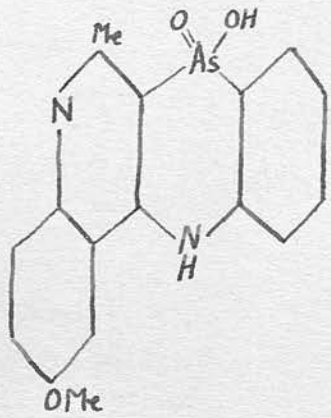
It has been shown by C.S. Gibson and his collaborators (J., 1927, 247, et seq.) that phenarsazine derivatives can be conveniently prepared from diphenylamine-o-arsonic acids by ring-closure under suitable conditions. Thus, for example, diphenylamine--o-arsonic acid (XXXVI), on treatment with boiling concentrated hydrochloric acid, is converted into phenarsazinic acid (XXXVII), which on reduction in alcohol-hydrochloric acid solution by means of sulphur dioxide in presence of a trace of iodine yields 10-chloro-5:10-dihydrophenarsazine (XXXVIII). This chlorodihydrophenarsazine can also be obtained directly from diphenylamine-o-arsonic acid (XXXVI) by reduction in alcohol-hydrochloric acid solution with sulphur dioxide in presence of iodine and is readily reconverted into phenarsazinic acid (XXXVII) on oxidation (compare Gibson and Johnson, J., 1927. 2501.).

It was therefore considered of interest to apply the above cyclisation methods to o-6'-methoxy-2'-methyl--4'-quinolylaminophenylarsonic acid (XIV) and o-5'--nitro-8'-quinolylaminophenylarsonic acid (XXXIV) with a view to obtaining the corresponding quinbenzarsazine derivatives.

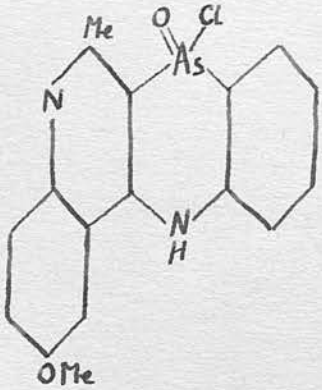
When/


Me
Cl
As
11
12
1
2
N 10
5
N
H
3
4
9
8
6
7
OMe

XXXIX.


Me
O
OH
As
N
N
H
OMe

XL


Me
O
Cl
As
N
N
H
OMe

XLI.

When o-6'-methoxy-2'-methyl-4'-quinolylamino-phenylarsonic acid (XIV) was boiled with concentrated hydrochloric acid for 4 hours, ring-closure did not take place and the arsonic acid was recovered unchanged. It was found however that, by reduction in boiling alcohol-hydrochloric acid solution by means of sulphur dioxide in presence of a trace of iodine, the arsonic acid (XIV) was readily converted into 12-chloro-7-methoxy-11-methyl-5:12-dihydroquinbenzarsazine (XXXIX). The halogen atom in this chlorodihydroquinbenzarsazine appears to be very stable and the compound can be recrystallised unchanged from hot water. This is very surprising in view of the fact that the chlorine atom in the simple chlorodihydrophenarsazines is highly labile (private communication from Professor C.S. Gibson). The abnormally low reactivity of the halogen atom in this quinoline derivative is referred to again below.

12-Chloro-7-methoxy-11-methyl-5:12-dihydroquinbenzarsazine was readily oxidised in acetic acid solution by means of hydrogen peroxide, and the product of the reaction proved to be 7-methoxy-11-methylquinbenzarsazinic acid (XL). This arsazinic acid possesses amphoteric properties (isoelectric point, pH 7.0), and its solutions in dilute acid and alkali exhibit a brilliant blue fluorescence. It can be readily re-converted/

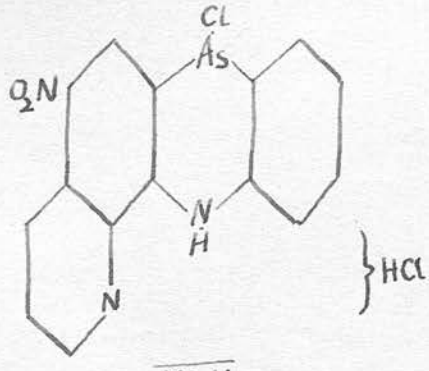
reconverted into the chlorodihydroquinbenzarsazine (XXXIX) by reduction in alcohol-hydrochloric acid solution with sulphur dioxide in the usual way.

Since ring-closure and formation of the quinbenzarsazinic acid (XL) did not take place on treatment of o-6'-methoxy-2'-methyl-4'-quinolylaminophenylarsonic acid (XIV) with boiling hydrochloric acid, it was considered of interest to ascertain whether cyclisation could be effected by other means. It was thought that this arsonic acid would be readily converted into the arsazinic acid by the action of phosphoryl chloride followed by decomposition of the product with water. The compound isolated from this experiment, however, proved not to be the free arsazinic acid since it was insoluble in cold dilute sodium hydroxide solution. It was found to contain chlorine which was not liberated by cold sodium hydroxide solution. This chlorocompound was soluble in acids, and the possibility that it was the hydrochloride of the arsazinic acid was excluded by the fact that these acid solutions exhibited no fluorescence. The elucidation of the constitution of this compound presented considerable difficulty until the observation was made that it dissolved slowly in boiling sodium hydroxide solution with simultaneous formation of free chloride ions to give a blue-fluorescing solution identical with that of

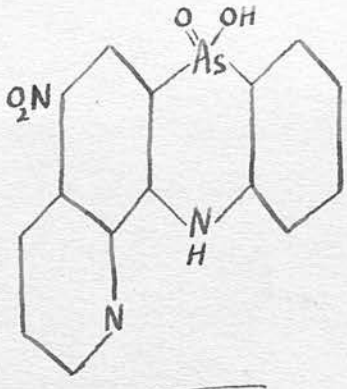
an/

an alkaline solution of the arsazinic acid (XL). This observation, taken in conjunction with the analytical results, indicated that this chloro-compound was 7-methoxy-11-methylquinbenzarsazinyl chloride (XLI). It has already been stated above that the chlorine atom in 12-chloro-7-methoxy-11-methyl-5:12--dihydroquinbenzarsazine (XXXIX) is surprisingly stable, and whilst it is remarkable that the chlorine atom in the arsazinyl chloride (XLI) is also stable, the two results are nevertheless consistent with each other and may have a common cause. It may be suggested that this cause is to be found in the proximity of the quinoline nitrogen atom, which would be expected to exert a positive charge on the arsenic atom and so prevent ionisation of the negatively charged chlorine atom.

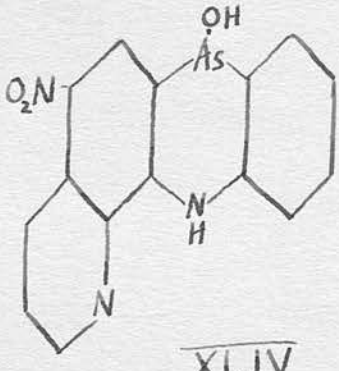
o-6'-Methoxy-2'-methyl-4'-quinolylaminophenyl-arsonic acid (XIV) dissolved readily in boiling acetic anhydride and the solution after decomposition with excess of water exhibited a brilliant blue fluorescence similar to that of an acetic acid solution of the arsazinic acid (XL). A similar blue fluorescence was also observed when a solution of the arsonic acid (XIV) in concentrated sulphuric acid was gently warmed. These reactions appear to indicate that ring-closure with formation of the arsazinic acid takes place readily by dehydrating agents such as acetic anhydride and/



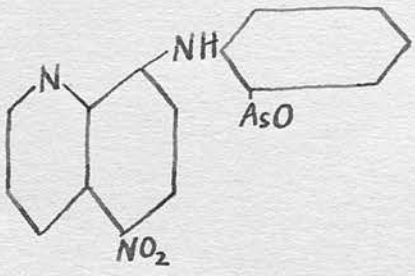
XLII



XLIII.



XLIV.



XLV.

and sulphuric acid.

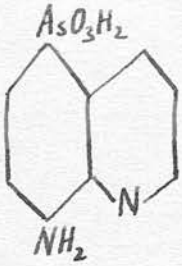
By reduction in boiling alcohol-hydrochloric acid solution by means of sulphur dioxide in presence of a trace of iodine, o-5'-nitro-8'-quinolylaminophenylarsonic acid (XXXIV) was readily converted into the dark red hydrochloride of 12-chloro-10-nitro-5:12-dihydroquinbenzarsazine (XLII), which on oxidation with hydrogen peroxide gave 10-nitroquinbenzarsazinic acid (XLIII). The sodium and potassium salts of this amphoteric acid show remarkable colour changes on dilution and addition of alkali. These colour reactions are similar to those described by Gibson and Johnson (J., 1929, 1254, 1262) in the case of certain nitrophenarsazinic acids in which the nitro-group occupies the same (meta-) position relative to the arsenic atom.

On treatment with four molecules of warm N/10-sodium hydroxide solution, the chlorine atoms in 12-chloro-10-nitro-5:12-dihydroquinbenzarsazine hydrochloride (XLII) were readily split off with production of an orange compound which is presumably 10-nitro-12-hydroxy-5:12-dihydroquinbenzarsazine (XLIV). This latter compound was also formed when the chloronitrodihydroquinbenzarsazine (XLII) was boiled with water for a few minutes. Compound XLII can be regenerated from 10-nitro-12-hydroxy-5:12-dihydroquinbenzarsazine in two ways (a) by the action of/

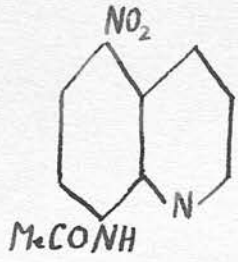
of boiling hydrochloric acid, (b) by oxidation with hydrogen peroxide in presence of acetic acid to the arsazinic acid (XLIII) and subsequent reduction in alcohol-hydrochloric acid solution with sulphur dioxide as described above. The fact that this chlorine-free compound (XLIV) can be oxidised to the arsazinic acid (XLIII) in presence of acetic acid appears to exclude the possibility that it possesses the arsen-oxide structure (XLV) since boiling acetic acid is itself unable to convert the open-ring arsonic acid (XXXIV) into this closed-ring arsazinic acid.

The ease with which the chlorine atoms in 12--chloro-10-nitro-5:12-dihydroquinbenzarsazine hydrochloride (XLII) are hydrolysed by boiling with water is of especial interest in view of the fact that the analogous 12-chloro-7-methoxy-11-methyl-5:12-dihydro--quinbenzarsazine (XXXIX) can be recrystallised unchanged from water. As has been mentioned above, it is possible that the reactivity of the chlorine atom in this latter compound may be influenced by the close proximity of the strongly polar quinoline nitrogen atom. In compound XLII the quinoline nitrogen atom is much farther removed from the halogen atom. This may have a bearing on the structure of these compounds which, for convenience, have been formulated in this thesis as dihydroarsazine derivatives without prejudice/

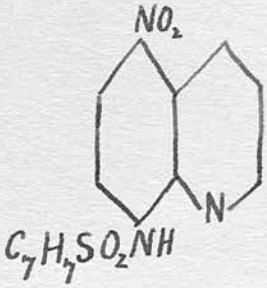
prejudice to the alternative arsazinium chloride formulation (compare Gibson and co-workers, J., 1929, 1238, et seq.; Kappelmeier, Rec. trav. chim., 1931, 50, 44).



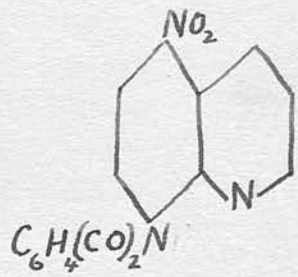
XLVI



XLVII



XLVIII



XLIX

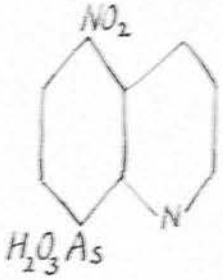
III. SYNTHESIS OF QUINOLINE DERIVATIVES CONTAINING AN ARSONIC ACID GROUP DIRECTLY ATTACHED TO THE QUINOLINE NUCLEUS.

Although certain of the more simple derivatives of quinoline containing an arsonic acid group directly attached to the nucleus can be readily obtained (compare Binz and Räth, Annalen, 1927, 453, 238; Fourneau, Tréfouel and Benoit, Ann. Inst. Pasteur, 1930, 44, 719; Balaban, J., 1930, 2346), the preparation of the quinoline analogues of many of the compounds of chemotherapeutic importance in the benzene series is unexpectedly difficult on account of the failure of the reactions employed in the latter case to give the desired results. For example, it was found that when 8-aminoquinoline and arsenic acid were heated together as in the Béchamp reaction condensation with formation of 8-aminoquinoline-5-arsonic acid (XLVI) did not take place and the original compounds were recovered unchanged except where extensive decomposition had set in on account of the prolonged heating at a high temperature. It may be mentioned here that Fränkel and Löwy (Ber., 1913, 46, 2546) attempted to arsonate quinoline and tetrahydroquinoline in the same way without success.

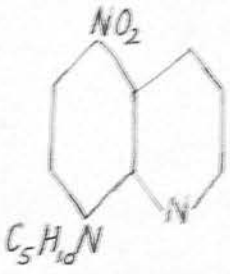
Since/

Since 8-aminoquinoline-5-arsonic acid could not be obtained in the above manner, attempts were made to prepare its acetyl derivative from 5-nitro-8--acetamidoquinoline (XLVII) [prepared from 5-nitro-8--aminoquinoline (XXXV); see p. 87] by reduction to 8-acetamido-5-aminoquinoline followed by replacement of the 5-amino-group by the arsonic acid group by means of the Bart reaction. However, owing to the ease with which 8-acetamido-5-aminoquinoline was oxidised on exposure to the air with formation of a black resinous product, it was not possible to isolate this compound in a pure condition. In order to stabilise the quinoline molecule, attempts were made to prepare 8-p-tolylsulphonamido-5-nitroquinoline (XLVIII) and 8-phthalimido-5-nitroquinoline (XLIX) by treatment of 5-nitro-8-aminoquinoline with p-tolylsulphonyl chloride and phthalic anhydride, respectively, with a view to reducing these compounds to the corresponding amino-derivatives, which might then be convertible into the arsonic acids by the Bart reaction.These nitroquinoline derivatives could not however be readily obtained. The explanation of these difficulties is probably to be found in the fact that 5-nitro-8-aminoquinoline is peculiar in that it possesses only very weakly basic properties (compare Dikshoorn, Rec. trav. chim., 1929, 48, 520).

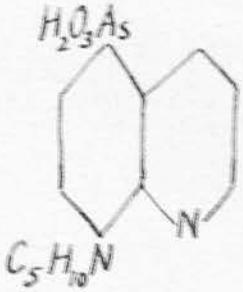
Since/



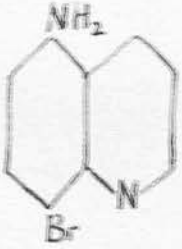
L



LI



LII



LIII

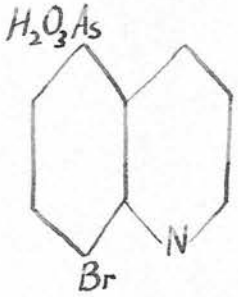
Since the halogen atom in 8-bromo-5-nitroquinoline (XXXIII) is highly reactive (compare p. 86), experiments were carried out with a view to preparing 5-nitroquinoline-8-arsonic acid (L) directly by heating the bromonitroquinoline with aqueous potassium arsenite in presence of ethyl alcohol and a trace of copper powder in an open vessel and also under pressure (compare Rosenmund, Ber., 1921, 54, 438; Gibson and Levin, J., 1931, 2399-2400). No success, however, attended any of these experiments.

When 8-bromo-5-nitroquinoline was heated with piperidine, condensation took place readily with the formation of 8-piperidino-5-nitroquinoline (LI), which on reduction gave 8-piperidino-5-aminoquinoline. Several attempts were then made to replace the amino-group in this latter compound by the arsonic acid group, but although the Bart reaction was carried out under various conditions it was not possible to isolate the desired 8-piperidinoquinoline-5-arsonic acid (LII). To circumvent this difficulty it was decided to investigate the possibility of preparing this piperidino--quinolylarsonic acid from 8-bromo-5-nitroquinoline by the introduction of the 5-arsonic acid group first followed by replacement of the bromine atom by the piperidino group.

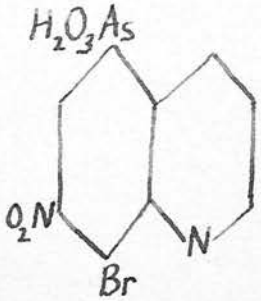
Claus and Howitz (J.pr. Chem., 1893, 48, 154), by reduction/

reduction of 8-bromo-5-nitroquinoline with stannous chloride and hydrochloric acid, obtained a base, m.p. 128-130° (only chloroplatinate analysed), which they considered to be 8-bromo-5-aminoquinoline (LIII) since when the amino-group was replaced by bromine by means of the Sandmeyer reaction it yielded 5:8-dibromo-:quinoline, m.p. 127°, as well as an unidentified product, m.p. 162°. Claus and Setzer, (J. pr. Chem., 1896, 53, 411) subsequently prepared a base, m.p. 136°, by the deacetylation of a compound, m.p. 250°, obtained by bromination of 5-acetamidoquinoline. The base, m.p. 136°, was considered to be 8-bromo-5-aminoquinoline in a pure form, the compound, m.p. 250°, being therefore orientated as 8-bromo-5-acetamidoquinoline.
It has now been found that when 8-bromo-5-nitroquinoline is reduced with iron filings in presence of alcoholic hydrochloric acid by West's method (J., 1925, 127, 494), a practically quantitative yield of base, m.p. 156-157°, is obtained. This base is almost certainly 8-bromo-5-aminoquinoline (LIII) and on acetylation it yields 8-bromo-5-acetamidoquinoline, m.p. 179-180°.
It is difficult to explain the discrepancy between these results and those of Claus and co-workers (above), but it may be noted that the analytical figures given by these investigators are very incomplete.

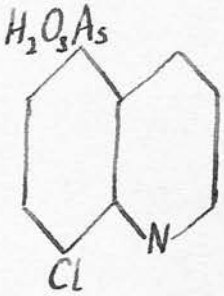
8-bromo-5-aminoquinoline was readily converted into/



LIV.



LV



LVI.

into 8-bromoquinoline-5-arsonic acid (LIV) by means of the Bart reaction particularly when the diazotised 8-bromo-5-aminoquinoline was allowed to react with sodium arsenite in acid solution rather than in alkaline solution. However, on treatment with boiling piperidine for 7 hours, the bromine atom in 8-bromo-quinoline-5-arsonic acid was found to be quite unreactive and no piperidinoquinolylarsonic acid (LII) was formed.

In order to mobilise the halogen atom in 8-bromo--quinoline-5-arsonic acid, attempts were made to prepare 8-bromo-7-nitroquinoline-5-arsonic acid (LV) by nitration. It was found, however, that no nitro-group could be introduced whether 8-bromoquinoline-5-arsonic acid was heated with (a) a mixture of sulphuric and nitric acids at 140° for 4 hours, (b) sulphuric acid containing potassium nitrate at 140° for 4 hours, (c) fuming sulphuric acid (10% SO_3) containing potassium nitrate at 95-100° for 10 hours. In all cases the sole product isolated proved to be unchanged initial material although oxidation tended progressively to decrease the yield.

Since several chloro-derivatives of benzene are more easily nitratable than the corresponding bromo-compounds, the possibility of introducing a nitro-group/

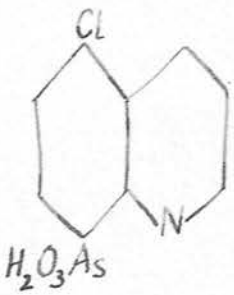
nitro-group into 8-chloroquinoline-5-arsonic acid (LVI) was investigated. This arsonic acid was prepared in the same way as its bromo-analogue by reduction of 8-chloro-5-nitroquinoline (prepared as described by Fourneau, Tréfouel and Wancolle, Bull. Soc. chim., 1930, 47, 740) to 8-chloro-5-aminoquinoline followed by replacement of the amino-group by the arsonic acid group. 8-Chloroquinoline-5-arsonic acid was found, however, to be just as unreactive as 8--bromoquinoline-5-arsonic acid (LIV) and no nitro-derivative could be prepared from it under the same nitration conditions used for the bromo-derivative (see p. 99).

In view of the fact that the simple 4-chloro- and 4-bromophenylarsonic acids are readily nitratable to give the corresponding 4-halogeno-3-nitrophenyl--arsonic acids (Barber, J., 1929, 473, 2337), it is very hard to understand why nitration of these 8-halogenoquinoline-5-arsonic acids should present such difficulties. A chloroquinolylarsonic acid capable of being nitrated to give a chloronitroquinolylarsonic acid containing a very reactive halogen atom was, however, synthesised in a rather unusual fashion as described below.

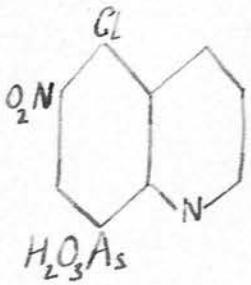
When 5-nitro-8-aminoquinoline (XXXV) was diazotised in presence of hydrochloric acid and then treated with sodium arsenite, the only pure product which /

which could be isolated from the reaction mixture proved to be a chloroquinolylarsonic acid and not the expected 5-nitroquinoline-8-arsonic acid (LIX). This chloro-compound must be either 5-chloroquinoline--8-arsonic acid (LVII) or, less likely, 8-chloro--quinoline-5-arsonic acid (LVI). The former compound would be formed if the first stage in this reaction is the introduction, in a normal manner, of the arsonic acid group into the quinoline nucleus in the 8-position. The 5-nitro-group must then be reduced by the excess of sodium arsenite to the amino-group, which is then diazotised by the small amount of free nitrous acid present, and the resulting diazonium group finally replaced by the chlorine atom from the hydrochloric acid. If the chloro-compound were 8-chloroquinoline-5-arsonic acid, then the reaction would consist in the replacement of the 8-amino-group by chlorine followed by reduction of the 5-nitro-group to the amino-group,which is then diazotised and the resulting diazonium group replaced by the arsonic acid group. That one or other of these two possibilities is correct is supported by the fact that the yield of this chloroquinolylarsonic acid was increased from about 10% to nearly 60% of the theoretical by the addition of a large excess of sodium arsenite and sodium nitrite to the diazotised 5-nitro-8-aminoquino-line mixture. This chloroquinolylarsonic acid (m.p.

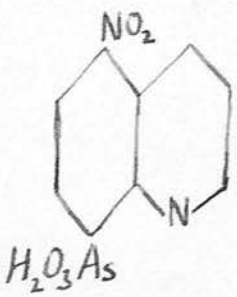
284/



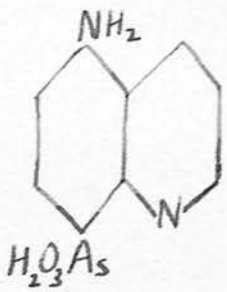
LVII



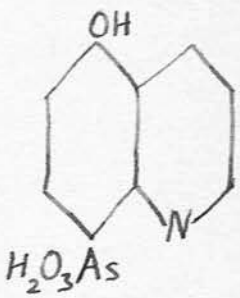
LVIII



LIX



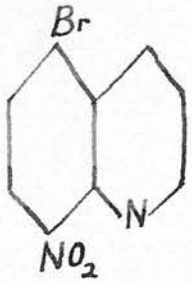
LX



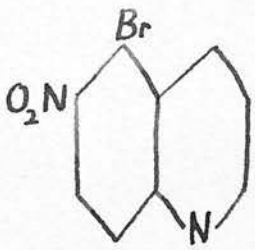
LXI

284-285°) was shown beyond all doubt to be different from 8-chloroquinoline-5-arsonic acid (m.p. 226-227°) since its melting-point was depressed to about 200° when it was mixed with an authentic specimen of the latter acid and also by the fact that, whilst it could not be nitrated at 140° with a mixture of sulphuric and nitric acids, it was slowly nitrated by means of a mixture of fuming sulphuric acid (10% SO_3) and potassium nitrate at 95-100°. These facts definitely exclude the second possibility mentioned above as to its formation. It follows that this chloroquinolyl--arsonic acid is in fact 5-chloroquinoline-8-arsonic acid (LVII). Since the chlorine atom in the nitro-derivative of this arsonic acid is very reactive, the nitro-group is without doubt attached to the quinoline nucleus in the 6-position and this nitro-compound is therefore 5-chloro-6-nitroquinoline-8-arsonic acid (LVIII).

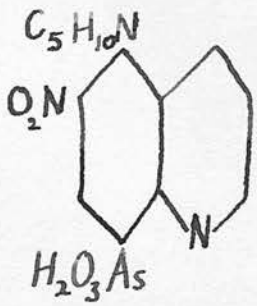
Since 5-chloroquinoline-8-arsonic acid was the only pure product which could be isolated by the application of the Bart reaction to 5-nitro-8-amino-quinoline in presence of hydrochloric acid, a series of experiments were carried out with diazo-solutions of this base in sulphuric or acetic acid. It was thought that 5-nitroquinoline-8-arsonic acid (LIX), 5-aminoquinoline-8-arsonic acid (LX) and 5-hydroxy-quinoline-8-arsonic acid (LXI) would be obtained by allowing/



LXII



LXIII.



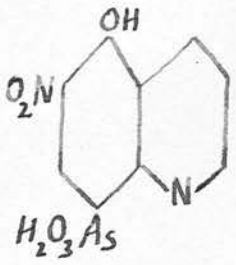
LXIV.

allowing these chloride-free diazo-solutions to react with (a) a small amount of sodium arsenite solution, (b) excess of sodium arsenite solution, and (c) excess of both sodium arsenite and sodium nitrite solutions, respectively. No success, however, attended these experiments and in all cases the only products obtained were dark brown compounds which were difficult to purify and, on analysis, were found to contain comparatively small amounts of arsenic. These deeply coloured brown compounds probably result from the interaction of quinoline diazonium salts with aminoquinolylarsonic acid formed by reduction of the intermediate nitroquinolylarsonic acid.

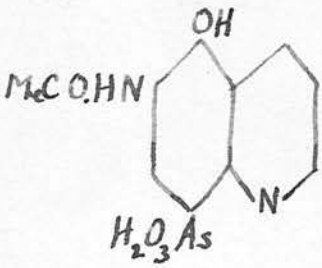
It is remarkable that 5-chloroquinoline-8-arsonic acid (LVII) can be nitrated whilst the isomeric 8-chloroquinoline-5-arsonic acid (LVI), as mentioned above, shows no tendency to nitrate under exactly the same conditions. These nitration results, however, may be compared with those of Dikshoorn (Rec. trav. chim., 1929, 48, 553, 556) (compare also Claus and Vis, J. pr. Chem., 1888, 38, 392; Claus and Howitz, ibid., 1893, 48, 153). Dikshoorn found that whilst 8-bromoquinoline nitrated to give only 8-bromo--5-nitroquinoline (XXXIII), 5-bromoquinoline gave a mixture of 12 parts of 5-bromo-8-nitroquinoline (LXII) and 1 part of 5-bromo-6-nitroquinoline (LXIII).

It is therefore not surprising to find that in the case of 8-bromo- and 8-chloroquinoline-5-arsonic acids no nitration takes place since the reactive 5-position is blocked. In 5-chloroquinoline-8-arsonic acid, on the other hand, the nitro-group may with difficulty be introduced into the 6-position, a result in keeping with the findings of Dikshoorn on the assumption that the arsonic acid group exerts at most a general damping effect on the molecule. It may be added that no one has succeeded in introducing two nitro-groups into either 8-bromoquinoline or 5-bromoquinoline. The damping effect of the nitro-group in any of these cases is apparently sufficient to prevent further attack. The very drastic treatment used by Dikshoorn (loc. cit. pp. 553-556) in attempts to di-nitrate these simple bromoquinolines was excluded in the case of the halogenoquinolylarsonic acids on account of the great ease with which they suffer complete oxidation. Fortunately, in the case of 5-chloroquinoline--8-arsonic acid, the nitration conditions mentioned above were sufficient to effect the desired result.

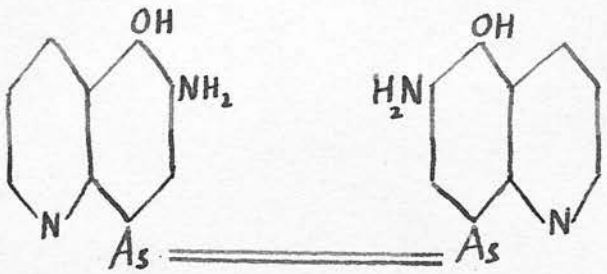
When 5-chloro-6-nitroquinoline-8-arsonic acid (LVIII) was heated on the water-bath with piperidine, condensation took place readily with elimination of hydrogen chloride and the formation of 5-piperidino--6-nitroquinoline-8-arsonic acid (LXIV). In the same way/



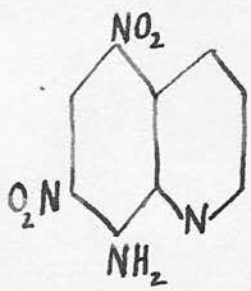
LXV



LXVI



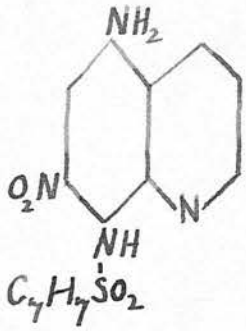
LXVII



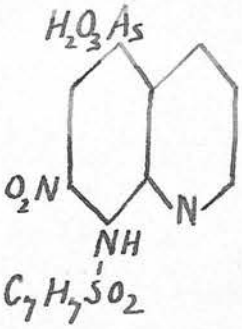
LXVIII

way 5-chloro-6-nitroquinoline-8-arsonic acid was converted on treatment with warm potassium hydroxide solution into 6-nitro-5-hydroxyquinoline-8-arsonic acid (LXV). The accessibility of this latter compound opens up a way for the synthesis of stovarsol and salvarsan analogues of quinoline with the formulae LXVI and LXVII respectively.

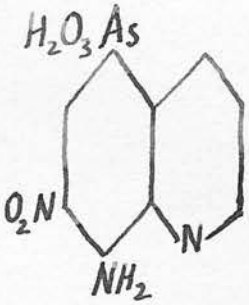
Since it was not possible to obtain either 8--bromo-7-nitroquinoline-5-arsonic acid (LV) or 8-chloro-7-nitroquinoline-5-arsonic acid by nitration of the corresponding 8-halogenoquinoline-5-arsonic acids (LIV and LVI), attempts were made to prepare these nitroquinolylarsonic acids in another way. Kaufmann and Zeller (Ber., 1917, 50, 1627) have shown that 5:7-dinitro-8-aminoquinoline (LXVIII) can be readily obtained from 8-p-tolylsulphonamidoquinoline by nitration to 8-p-tolylsulphonamido-5:7-dinitro--quinoline followed by removal of the p-tolylsulphonyl group by means of warm sulphuric acid. It was thought that 5:7-dinitro-8-aminoquinoline might be convertible into 8-bromo-5:7-dinitroquinoline and 8-chloro-5:7--dinitroquinoline by the Sandmeyer reaction. These halogeno-compounds might then be reducible with ammonium sulphide to give the corresponding 8-halogeno--7-nitro-5-aminoquinolines, which could then be converted/



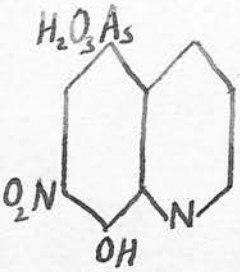
LXIX



LXX.



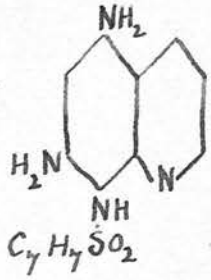
LXXI



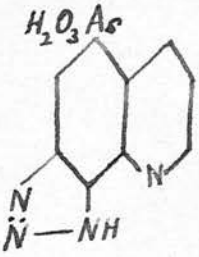
LXXII.

converted into the required arsonic acids by means of the Bart reaction. It was found, however, that 5:7-dinitro-8-aminoquinoline, which is practically devoid of basic properties (compare Dikshoorn, loc. cit., p.525), did not diazotise under the conditions usually employed and so it could not be made to undergo the Sandmeyer reaction. It may be noted here that Dikshoorn (loc. cit., p.556) obtained 8-chloro-5:7-dinitroquinoline by treatment of 5:7-dinitro-8-hydroxyquinoline with p-tolylsulphonyl chloride. The yield, however, was very small and this method is obviously unsuitable for the preparation of the chloro-compound in sufficient quantity to enable the above scheme to be investigated.

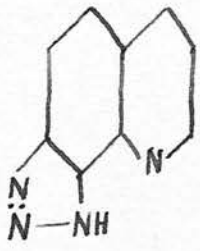
Experiments were then carried out with a view to reducing 8-p-tolylsulphonamido-5:7-dinitroquinoline by means of ammonium sulphide to 8-p-tolylsulphonamido--7-nitro-5-aminoquinoline (LXIX), which should then be convertible into 8-p-tolylsulphonamido-7-nitroquinoline-5-arsonic acid (LXX), and which in turn would yield 7-nitro-8-aminoquinoline-5-arsonic acid (LXXI). This latter compound could then be converted into 7-nitro-8-hydroxyquinoline-5-arsonic acid (LXXII), which should yield salvarsan and stovarsol analogues of quinoline isomeric with those formulated on p. 105A. However, it was found that reduction of 8-p-tolyl--sulphonamido-5:7-dinitroquinoline by means of ammonium sulphide/



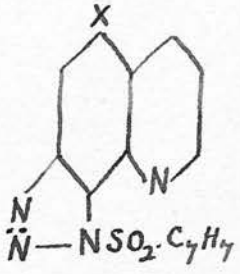
LXXIII



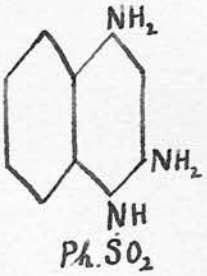
LXXIV



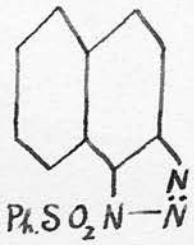
LXXV



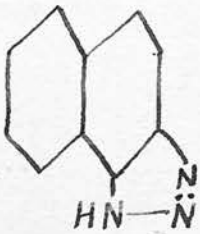
LXXVI



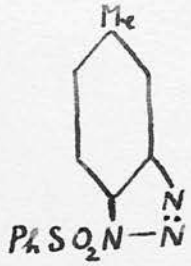
LXXVII



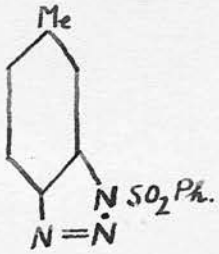
LXXVIII



LXXIX



LXXX



LXXXI

sulphide gave a practically quantitative yield of 8-p-tolylsulphonamido-5:7-diaminoquinoline (LXXIII). When this compound was treated with two molecules of sodium nitrite in presence of hydrochloric acid, a pronounced odour of p-tolylsulphonyl chloride was produced, and when the resulting diazo-solution was allowed to react with sodium arsenite solution two compounds were formed, namely 7:8-triazolquinoline-5-arsonic acid (LXXIV) and 7:8-triazolquinoline (LXXV). The absence of a group in the 5-position in the latter compound is presumably to be explained as due to the reduction of the intermediate 5-diazonium group by means of arsenious acid. The condensed heterocyclic ring structure contained in these two triazol compounds does not appear previously to have been synthesised, and 7:8-triazolquinoline is in fact the simplest representative of this new system.

The facile removal of the p-tolylsulphonyl group by the action of nitrous acid under the above conditions is very remarkable especially when it is realised that it is possible for ring-closure to take place without the elimination of this group so as to yield compound LXXVI. It is interesting to note, however, that Morgan and Godden (J., 1910, 97, 1717) observed that, when the analogous 4-phenylsulphonamido--1:3-diaminonaphthalene (LXXVII) was diazotised in presence of alcohol, deacylation tended to take place

to some extent, and the phenylsulphonyltriazol compound (LXXVIII) produced was always contaminated with a certain amount of the simple triazol derivative (LXXIX). It has also been shown that the acyl group in certain of these triazol derivatives shows a marked tendency to migrate from one nitrogen atom to another. Thus, for example, the triazol compound LXXX can be readily converted by the action of boiling alcohol or benzene into the isomeric compound LXXXI (compare Morgan and Scharff, J., 1914, 105, 117). The results recorded above may be regarded as an extreme case of this mobility in which complete elimination instead of migration of the p-tolylsulphonyl group takes place. The dissociated p-tolylsulphonyl kation appears to have combined with the chloride anion from the hydrochloric acid, whilst the negatively charged triazolquinolyl ion has united with the hydrogen ion.
It is possible that the influence of the quinoline nitrogen atom, which is positively charged in acid solution, is to increase the tendency of the positively charged p-tolylsulphonyl group to ionise completely with the result that the double decomposition indicated above is effected.

The above quinoline derivatives of arsenic and some of the intermediate compounds are being tested by/

by the Chemotherapy Committee of the Medical Research Council in respect of their chemotherapeutic actions in malaria and trypanosomiasis.

EXPERIMENTAL.

4-p-Aminoanilino-6-methoxy-2-methylquinoline (XVI).

A mixture of 4-chloro-6-methoxy-2-methylquinoline (10.4 g.) (prepared as described by Conrad and Limpach, Ber. 1888, 21, 1651) and p-phenylenediamine (9 g.) was heated at 130-140°/15 mm. during 20 minutes. The mixture became dark brown and gradually more viscous and at the end of the period mentioned had almost completely solidified. The reaction product was dissolved in the minimum amount of hot dilute hydrochloric acid (5%), filtered, cooled, and added to twice its volume of concentrated hydrochloric acid (d 1.19). After a few minutes a pale green, crystalline hydrochloride was precipitated. This was filtered off, washed with absolute alcohol (yield, 13.5 g.), and converted into the free base by the addition of excess of ammonium hydroxide to its aqueous solution. The base was purified by recrystallisation from aqueous alcohol (50%) from which it separated in large, thick, light brown, rectangular plates, m.p. 215° with slight frothing. This frothing appears to be due to the loss of a small quantity of water of crystallisation and was observed even after prolonged heating of the/

the crystals at 100° (Found: C, 68.5; H, 6.2. $C_{17}H_{17}ON_3,H_2O$ requires C, 68.7; H, 6.4%).

4-p-Aminoanilino-6-methoxy-2-methylquinoline is soluble in ethyl alcohol, methyl alcohol, and acetic acid, but much less soluble in benzene and light petroleum. The hydrochloride separates in fine, white prismatic needles when hydrochloric acid is added to an alcoholic solution of the base. A dilute alcoholic or acetic acid solution of the base does not give a coloration with an N/1000-solution of iodine in aqueous potassium iodide.

The acetyl derivative of the above base, prepared by means of boiling acetic anhydride, crystallised from aqueous alcohol (20% alcohol) in large, pale yellow rhombohedra which contained water of crystallisation. After being heated at 150° for 4 hours the compound had m.p. 240° (Found: C, 71.2; H, 5.7; N, 12.8. $C_{19}H_{19}O_2N_3$ requires C, 71.0; H, 6.0; N, 13.1%). It is soluble in alcohol and acetic acid, but practically insoluble in benzene and light petroleum. Hydrochloric acid precipitates the hydrochloride from an alcoholic solution in yellow feathery needles which are sparingly soluble in water. A warm dilute acetic acid solution of the acetyl compound (2%) sets on cooling to a firm jelly. A dilute alcoholic or acetic acid solution gives a brilliant blue coloration with an N/1000-solution of iodine in aqueous potassium iodide/

iodide. This coloration disappears on warming and in the case of the acetic acid solution reappears on cooling. Fresh iodine solution, however, has to be added to the cooled dilute alcoholic solution in order to restore the blue colour. A blue coloration is also produced when a dilute aqueous solution of potassium iodide is added to a mixture of an acetic acid solution of the acetyl compound and bromine water.

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p-6'-Methoxy-2'-methyl-4'-quinolylaminophenylarsonic Acid (XV).

4-p-Aminoanilino-6-methoxy-2-methylquinoline (5.6 g.) was added to hydrochloric acid (9 cc.; d 1.19) and the mixture cooled to -5° during constant stirring. A solution of sodium nitrite (1.6 g.) in water (7 cc.) was then added gradually, care being taken to prevent the temperature rising above 0°. The mixture was allowed to stand for one hour and then cautiously neutralised at 0° with aqueous sodium hydroxide (5N). Sodium arsenite solution (a mixture of arsenious oxide, 3 g., in 5N-sodium hydroxide solution, 6 cc.; sodium carbonate, 6 g., in water, 18 cc.; and 10% copper sulphate solution, 0.6 cc., to which was added sufficient aqueous ammonium hydroxide to give the soluble complex salt) was then added. Nitrogen was slowly evolved with production of a copious froth, and the mixture was allowed to stand at room-temperature for 16 hours. It was then warmed gently on the water-bath until evolution of nitrogen ceased, filtered, and the solid residue extracted thrice with small amounts of aqueous sodium hydroxide (5%). The hydrogen-ion concentration of the combined filtrates was adjusted with hydrochloric acid to pH 7.5; the arsonic acid was then quantitatively precipitated as a white, gelatinous/

gelatinous solid. This was collected, redissolved in dilute sodium hydroxide solution and reprecipitated at its isoelectric point (pH 7.5), the process being repeated several times. It was then filtered off and washed thoroughly with distilled water. When nitric acid was used instead of hydrochloric acid to precipitate the arsonic acid from its solution in dilute sodium hydroxide solution, the arsonic acid separated in the form of stellate clusters of fine, white, prismatic needles. The acid was unmolten at 307° (Found: N, 7.6; As, 19.4. $C_{17}H_{17}O_4N_2As$ requires N, 7.2; As, 19.3%). The yield of arsonic acid obtained by this method ranged from 0.5 to 3.2 g.. It was found that a more uniform yield of material could be obtained by isolating the solid diazonium compound and allowing it to react with sodium arsenite mixture as follows.

4-p-Aminoanilino-6-methoxy-2-methylquinoline (5.6 g.) was added to glacial acetic acid (30 cc.) and the well-stirred mixture cooled to 10°. Sodium nitrite (1.6 g.) was then added gradually and when diazotisation was complete the clear reddish-brown solution was poured into dry ether (150 cc.). The solid orange diazonium compound, which separated, was filtered off, washed with dry ether and then added to the sodium arsenite mixture (as used above).

After/

After standing overnight, the reaction product was worked up and the arsonic acid isolated as in the last experiment (yield, 2.6 g).

p-6'-Methoxy-2'-methyl-4'-quinolylaminophenylarsonic acid is practically insoluble in water and in the usual organic solvents. It dissolves readily in dilute sodium and ammonium hydroxide solutions and in moderately concentrated hydrochloric acid. No satisfactory explanation can be advanced of the curious phenomenon that the acid is precipitated from a solution of its sodium salt in a crystalline form by nitric acid (vide supra) and, under exactly similar conditions, in a gelatinous form by hydrochloric acid. A colloidal solution of the arsonic acid in dilute acetic acid gives a purple coloration with N/1000-iodine.

The sodium salt is precipitated in colourless plates when concentrated sodium hydroxide solution is added to a solution of the arsonic acid in dilute alkali. The following salts are precipitable from an aqueous solution of the ammonium salt: magnesium salt, white, gelatinous, insoluble in hot water; barium salt, white, gelatinous, soluble in hot water, crystallises on cooling in fine slender needles; calcium salt, white, gelatinous, less soluble in hot water; silver salt, pale yellow, curdy, insoluble in hot water; mercuric salt, white, curdy, insoluble in hot water.

4-m-Aminoanilino-6-methoxy-2-methylquinoline (XVII).

A mixture of 4-chloro-6-methoxy-2-methylquinoline (10.4 g.) and m-phenylenediamine (10 g.) was heated at 140°/20 mm. for 1½ hours. The melt slowly thickened and at the end of the period stated had almost completely solidified. The filtered solution of the product in hot dilute hydrochloric acid (5%) was rendered alkaline with ammonium hydroxide, and the light brown precipitate of the base which separated was purified by repeated recrystallisation from aqueous alcohol (20% alcohol) from which it was finally obtained in long, pale brown, rectangular, prismatic needles, m.p. 230-231° (yield, 10 g.) (Found: C, 73.4; H, 6.2; N, 14.7. $C_{17}H_{17}ON_3$ requires C, 73.1; H, 6.1; N, 15.0%).

4-m-Aminoanilino-6-methoxy-2-methylquinoline is readily soluble in ethyl alcohol, methyl alcohol, and acetic acid, but sparingly soluble in benzene and light petroleum. The addition of hydrochloric acid to an alcoholic solution of the base gives a white gelatinous precipitate of the hydrochloride.
A dilute alcoholic or acetic acid solution of the base gives a faint red coloration with an N/1000-solution of iodine in aqueous potassium iodide. This coloration deepens slowly on standing.

The/

The acetyl derivative of the above base, prepared by means of boiling acetic anhydride in the usual way, crystallised from aqueous alcohol (10% alcohol) in stout, pale yellow, prismatic needles, m.p. 269° (Found: C, 71.0; H, 6.1; N, 12.7. $C_{19}H_{19}O_2N_3$ requires C, 71.0; H, 6.0; N, 13.1%). It is slightly soluble in ethyl alcohol and acetic acid, but much less soluble in benzene and light petroleum. It dissolves in concentrated hydrochloric acid and the addition of water to this solution produces a white, gelatinous precipitate of the hydrochloride. A dilute alcoholic solution of the acetyl compound gives a deep blue coloration with an N/1000-solution of iodine in aqueous potassium iodide, whilst a dilute acetic acid solution, under the same conditions, gradually gives a brilliant purple coloration; these colours disappear on warming but reappear on cooling.

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m-6'-Methoxy-2'-methyl-4'-quinolylaminophenylarsonic Acid (XVIII).

This arsonic acid was obtained from 4-m-amino-anilino-6-methoxy-2-methylquinoline by the Bart reaction under practically the same conditions which were used in the first method of preparation of its p-isomeride (vide supra). The base (5.6 g.) was added to hydrochloric acid (15 cc.; d 1.14) and the mixture cooled to 0° during constant stirring. A solution of sodium nitrite (1.6 g.) in water (7 cc.) was then added at such a rate that the temperature of the mixture did not rise above 5°. After all the sodium nitrite solution had been added the mixture was allowed to stand for 1 hour. It was then cautiously neutralised with 5 N-sodium hydroxide solution and sodium arsenite solution (as used on p.113) added; nitrogen was then slowly evolved. After standing overnight at room-temperature, the mixture was carefully warmed on the water-bath until evolution of nitrogen ceased, filtered, and the reaction of the alkaline filtrate adjusted with hydrochloric acid to pH 5; the arsonic acid was then quantitatively precipitated as a white, gelatinous solid. This was filtered off and purified by several reprecipitations from its solution in dilute aqueous sodium hydroxide at the above isoelectric point (pH 5). It was then washed/

washed with distilled water and dried (yield, 1.5 g.). The acid darkened at 280° but was unmolten at 300°. (Found: As, 19.5. $C_{17}H_{17}O_4N_2As$ requires As, 19.3%).

m-6'-Methoxy-2'-methyl-4'-quinolylaminophenylarsonic acid is practically insoluble in water and in the usual organic solvents. It dissolves readily in dilute sodium and ammonium hydroxide solutions and in moderately concentrated hydrochloric acid. Unlike its p-isomeride this arsonic acid is not precipitated in a crystalline form when nitric acid is added to an aqueous solution of its sodium salt (see p. 114). A colloidal solution of the arsonic acid in dilute acetic acid does not give a coloration with an N/1000-solution of iodine in aqueous potassium iodide.

The sodium salt of the arsonic acid is readily precipitated as a crystalline mass when concentrated sodium hydroxide solution is added to its dilute alkali solution. The following salts are insoluble in cold water: magnesium salt, white, gelatinous, slightly soluble in hot water; calcium salt, pale yellow, gelatinous, less soluble in hot water; barium salt, white, gelatinous, soluble in hot water, gelatinous precipitate on cooling; silver salt and mercuric salt, pale yellow, curdy, insoluble in hot water.

4-Benzidino-6-methoxy-2-methylquinoline (XXI).

A mixture of 4-chloro-6-methoxy-2-methylquinoline (5.2 g.) and benzidine (7 g.) was heated at 160° during 15 minutes. The brown melt rapidly became viscous, and at the end of the period mentioned had almost completely solidified. The solid was digested with excess of hot dilute hydrochloric acid (5%), which readily converted it into an insoluble yellow hydrochloride. This was extracted several times with small quantities of boiling dilute hydrochloric acid in order to remove any unchanged chloroquinoline and benzidine, and then converted into the free base (7.2 g.) by the action of warm sodium hydroxide solution. The crude base was purified by recrystallisation from toluene and separated in fine, pale yellow, microscopic, rectangular prisms, m.p. 245° (Found: C, 77.8; H, 6.1; N, 11.5. $C_{23}H_{21}ON_3$ requires C, 77.7; H, 6.0; N, 11.8%).

4-Benzidino-6-methoxy-2-methylquinoline is moderately easily soluble in acetic acid and boiling toluene, but only sparingly soluble in ethyl and methyl alcohols, benzene and light petroleum. Its solution in warm dilute acetic acid sets to a gel on cooling. A dilute acetic acid solution of the base gives a deep purple coloration with an N/1000-solution/

solution of iodine in aqueous potassium iodide. This colour disappears on warming but does not reappear on cooling.

The acetyl derivative of the above base, prepared by means of boiling acetic anhydride in the usual way, was purified by recrystallisation from aqueous alcohol and separated on cooling in stellate clusters of fine, yellow, rectangular, prismatic needles, m.p. 159-160° (Found: N, 9.7; $C_{25}H_{23}O_2N_3,2H_2O$ requires N, 9.7%). It is readily soluble in ethyl and methyl alcohols and in acetic acid, but it is only slightly soluble in benzene and practically insoluble in light petroleum. The addition of hydrochloric acid to its solution in ethyl alcohol precipitates a crystalline hydrochloride, which is sparingly soluble in water. A solution of the base in warm dilute acetic acid sets to a gel on cooling. A dilute alcoholic or acetic acid solution gives with N/1000-iodine a brilliant purple coloration, which disappears on warming and reappears on cooling. This coloration is also produced when a dilute aqueous potassium iodide solution is added to a mixture of an acetic acid solution of the acetyl compound and bromine water.

4'-6"-Methoxy-2"-methyl-4"-quinolylaminodiphenylyl-arsonic Acid (XXII).

4-Benzidino-6-methoxy-2-methylquinoline (3.5 g.) was added to dilute hydrochloric acid (5.7 cc. of hydrochloric acid, d 1.19; 15 cc. of water) and the mixture cooled to 5° during constant stirring. A saturated solution of sodium nitrite (0.8 g.) in water was then added gradually so that the temperature was kept below 8°. After all the sodium nitrite had been added, the mixture was allowed to stand for 1 hour and then carefully neutralised at 5° with aqueous sodium hydroxide (5N). Sodium arsenite solution (a mixture of arsenious oxide, 2 g., in 5N-sodium hydroxide solution, 4 cc.; sodium carbonate, 4 g., in water, 12 cc.; and 10% copper sulphate solution, 0.4 cc.; to which was added sufficient aqueous ammonium hydroxide solution to give the soluble complex salt) was then added. The mixture was allowed to stand at room-temperature overnight and then warmed gently on the water-bath until evolution of nitrogen ceased. After the addition of N-sodium hydroxide solution (10 cc.), the liquid was filtered and the residue extracted thrice with warm dilute sodium hydroxide solution. The reaction of the combined filtrates was adjusted with hydrochloric acid in the cold to pH/

pH 7; the arsonic acid was then completely precipitated as a light brown, flocculent solid. Purification was effected by several reprecipitations (at pH 7) from its solution in dilute alkali with hydrochloric acid, and the arsonic acid was finally obtained in pale yellow needles (radially arranged) which, when washed free from sodium chloride, were converted into a gelatinous solid (yield, 2.2 g.). The acid darkened at 290° but was unmolten at 300° (Found: As, 16.0. $C_{23}H_{21}O_4N_2As$ requires As, 16.15%).

4'-6"-Methoxy-2"-methyl-4"-quinolylaminodiphenylyl-arsonic acid is practically insoluble in water and in the usual organic solvents. It is readily soluble in dilute sodium and ammonium hydroxide solutions but only slightly soluble in hydrochloric acid. A crystalline precipitate of the sodium salt is readily produced when concentrated sodium hydroxide solution is added to the solution of the arsonic acid in dilute alkali. The following insoluble metallic salts of the arsonic acid are obtained by the addition of a soluble salt of the corresponding metal to an aqueous solution of the ammonium salt of the acid: magnesium salt; white, gelatinous, insoluble in hot water: calcium salt; white, gelatinous, insoluble in hot water: barium salt; white, gelatinous, slightly soluble in hot water, amorphous precipitate on cooling: silver salt; pale yellow, gelatinous, insoluble/

insoluble in hot water: mercuric salt; pale yellow, curdy, insoluble in hot water. A colloidal solution of the arsonic acid in dilute acetic acid gives a dark red coloration with an N/1000-solution of iodine in aqueous potassium iodide. This colour changes to purple on the addition of hydrochloric acid.

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pp'-Di-6-methoxy-2-methyl-4-quinolyldiaminodiphenylmethane (XXIII).

pp'-Diaminodiphenylmethane (7.5 g.) was heated to 130°, and to the melt was added 4-chloro-6-methoxy-2-methylquinoline (5.2 g.) along with a trace of finely divided copper-bronze. The mixture darkened slightly and gradually became more viscous, and after being heated for 3 hours at the above temperature it had almost completely solidified. It was then digested with hot dilute hydrochloric acid (5%) which dissolved out unchanged diaminodiphenylmethane and chloroquinoline and converted the condensation product into a pale yellow hydrochloride which was sparingly soluble in water. This was collected (4.5 g.) and converted into the free base by the addition of warm aqueous sodium hydroxide solution. The base was purified by recrystallisation from hot alcohol and separated on cooling in small, pale brown, rectangular, prismatic needles, m.p. 144° (decomp.) (Found: C, 77.3; H, 6.0; N, 10.2. $C_{35}H_{32}O_2N_4$ requires C, 77.7; H, 6.0; N, 10.4%).

pp'-Di-6-methoxy-2-methyl-4-quinolyldiaminodiphenylmethane is moderately easily soluble in ethyl alcohol and acetic acid, but only sparingly soluble in benzene and light petroleum. The addition of hydrochloric/

hydrochloric acid to an alcoholic solution of the base precipitates the hydrochloride in fine yellow rectangular prismatic needles. A dilute alcoholic or acetic acid solution of the base gives with N/1000-iodine a deep purple coloration, which disappears on warming but reappears on cooling.

In experiments in which 4-chloro-6-methoxy-2-methylquinoline (2.6 g.) and pp'-diaminodiphenylmethane (up to 10 g.) were heated together at 130° for 4 hours the diaminodiphenylmethane was in every case partially converted into the above diquinolyl derivative and no trace of the monoquinolyl compound could be obtained.

4-Bromo-6-methoxy-2-methylquinoline (XXV).

4-Hydroxy-6-methoxy-2-methylquinoline was obtained in good yield by the following modification of the method of Conrad and Limpach (Ber., 1888, 21, 1649).

p-Anisidine (61.5 g.) was dissolved in ethyl acetoacetate (65 g.) at 37°, and the mixture maintained at this temperature during 3 days. The water, which had separated at the end of this time, was removed under diminished pressure at 60°, and the ethyl β-p-methoxyphenylaminocrotonate formed was heated very rapidly to 250-260° in a flask fitted with a side-tube and condenser. The flame was removed whenever the vigorous reaction, which took place at this temperature, began to subside, and the light brown residue, which solidified readily on cooling, was repeatedly extracted with hot dilute hydrochloric acid (5%) until a test portion gave no precipitate with ammonium hydroxide. The combined extracts were boiled with animal charcoal for a few minutes, filtered, and rendered alkaline with ammonium hydroxide solution. The pale yellow crystalline precipitate of 4-hydroxy-6-methoxy-2-methylquinoline, which separated, was then washed with water and dried at 100°, m.p. 296-298° with slight decomposition; yield, 57.5 g. (61% of the theoretical): Conrad and Limpach (loc. cit.,/

cit., p.1650) obtained a 37% yield.

4-Hydroxy-6-methoxy-2-methylquinoline (25 g.) was added to phosphoryl bromide (50 g.), and the mixture was vigorously shaken at 80-90° for 1 hour. The product was then decomposed with water (400 cc.) at 60°, the resulting dark brown solution boiled with animal charcoal for 10 minutes, filtered, and rendered alkaline with sodium hydroxide solution. The dark brown flocculent base, which separated was collected, washed with water and dried. It recrystallised from light petroleum (b.p. 60-80°) in sheaves of fine, pale yellow, rectangular, prismatic needles, m.p. 117-118°; yield, 15.5 g. (Found: C, 52.5; H, 4.2; N, 5.3. $C_{11}H_{10}ONBr$ requires C, 52.4; H, 4.0; N, 5.6%). The rather poor yield is due to demethylation of the methoxyl group by the liberated hydrobromic acid.

4-Bromo-6-methoxy-2-methylquinoline is slightly soluble in hot water. It dissolves readily in ethyl alcohol, methyl alcohol, ethyl acetate, acetone, ether, chloroform, benzene, and acetic acid, but it is much less soluble in ligroin and light petroleum. The acetic acid solution exhibits a brilliant blue fluorescence. It is readily soluble in dilute mineral acids. Its solution in dilute sulphuric acid exhibits a greenish-blue fluorescence, which changes to violet on further dilution. A dilute nitric acid solution exhibits a blue fluorescence, but no

fluorescence/

fluorescence is observed with a solution in dilute hydrochloric acid. The addition of a drop of sulphuric or nitric acid to the hydrochloric acid solution of the base produces only a faint fluorescence, and the intensity of the fluorescences in the dilute sulphuric and nitric acid solutions is greatly diminished by the addition of hydrochloric acid. A dilute alcoholic or acetic acid solution of the base does not give a coloration with an N/1000-solution of iodine in aqueous potassium iodide.

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o-6'-Methoxy-2'-methyl-4'-quinolylaminophenylarsonic Acid (XIV).

4-Bromo-6-methoxy-2-methylquinoline (8.5 g.) and o-aminophenylarsonic acid (5.4 g.) were dissolved in perfectly dry, warm amyl alcohol (70 cc.), and powdered anhydrous potassium carbonate (16 g.) and traces of finely divided copper-bronze and iodine added. The mixture was maintained at 130-140° in an oil-bath during 22 hours. It was then cooled, and the amyl alcohol removed by distillation in steam. The residual dark reddish-brown aqueous solution was boiled for a few minutes with animal charcoal, filtered, and the solid residue extracted thrice with small quantities of warm dilute aqueous sodium hydroxide solution. The hydrogen-ion concentration of the combined filtrates was adjusted with hydrochloric acid to pH 7.3, and the arsonic acid thereby quantitatively precipitated as a light brown, gelatinous solid, was purified by several reprecipitations from its solution in warm dilute aqueous sodium hydroxide (animal charcoal) at its isoelectric point (pH 7.3) with hydrochloric acid. The white, gelatinous solid thus obtained was filtered off, washed with distilled water, and dried at 100°, m.p. 302-303° (decomp.) (yield, 5.9 g.) (Found: As,/

As, 19.3. $C_{17}H_{17}O_4N_2As$ requires As, 19.3%).

o-6'-Methoxy-2'-methyl-4'-quinolylaminophenylarsonic acid is practically insoluble in water and in the usual neutral organic solvents, but it is readily soluble in acetic acid and in a mixture of alcohol and hydrochloric acid. A saturated solution in hot dilute acetic acid deposits well-shaped, colourless rhombic prisms on cooling. The acid dissolves readily in dilute sodium and ammonium hydroxide solutions and in moderately concentrated hydrochloric acid. A colloidal solution of the arsonic acid does not give a coloration with an N/1000-solution of iodine in aqueous potassium iodide. If, however, a few drops of concentrated hydrochloric acid are added to the mixture a brilliant violet coloration appears: this disappears on the addition of excess of hydrochloric acid. The arsonic acid dissolves readily in boiling acetic anhydride to give a yellow solution which when added to excess of water exhibits a brilliant blue fluorescence.
A solution of the arsonic acid in warm concentrated sulphuric acid exhibits a similar blue fluorescence which persists on dilution with water. No fluorescence is produced with a hydrochloric acid solution of the arsonic acid even on prolonged boiling.

The sodium salt is precipitated in stellate clusters of fine, colourless, prismatic needles when concentrated/

concentrated sodium hydroxide solution is added to a solution of the arsonic acid in dilute alkali. The following salts are precipitable from an aqueous solution of the ammonium salt: magnesium salt, pale yellow, amorphous, insoluble in hot water; barium salt, fine feathery needles, moderately easily soluble in cold water, readily soluble in hot water; silver salt, pale yellow, amorphous, insoluble in hot water: mercuric salt, white, curdy, insoluble in hot water. The calcium salt appears to be readily soluble. It is slowly precipitated, however, in sheaves of fine slender needles when excess of a concentrated calcium chloride solution is added to the ammonium salt solution.

4-o-Tolidino-6-methoxy-2-methylquinoline (XXVI).

A mixture of 4-bromo-6-methoxy-2-methylquinoline (5.0 g.) and o-tolidine (10 g.) was heated at 140-150° in an oil-bath during 9 hours. The melt gradually became more viscous, and at the end of the period stated had almost completely solidified. The reaction product was digested with excess of boiling dilute hydrochloric acid (10%) which readily converted it into a practically insoluble light brown hydrochloride. This was collected, washed with dilute hydrochloric acid (10%), and converted into the free base (7.2 g.) by means of warm aqueous sodium hydroxide solution. It recrystallised from aqueous alcohol (40% alcohol) in fine pale yellow rhombohedra, which melted at 152° with loss of solvent of crystallisation. After being heated for 5 hours at 100°, the compound melted at 199-200° (Found: C, 78.3; H, 6.7; N, 10.9. $C_{25}H_{25}ON_3$ requires C, 78.3; H, 6.6; N, 11.0%).

4-o-Tolidino-6-methoxy-2-methylquinoline is insoluble in water. It is readily soluble in chloroform and acetone, moderately easily soluble in ethyl alcohol, methyl alcohol, ethyl acetate and acetic acid, sparingly soluble in ether, benzene, and ligroin, and practically insoluble in light petroleum. It is slightly soluble in boiling dilute hydrochloric acid and,/

and, on cooling, the hydrochloride crystallises in colourless octahedra. The sulphate is deposited from solution in warm dilute sulphuric acid in a white gelatinous mass. The base dissolves in dilute nitric acid to give a brown solution. A solution of the diazo-chloride gives a blood-red azo-dye with an alkaline solution of β-naphthol. A dilute solution of the base in aqueous alcohol or acetic acid gives a reddish-brown coloration with an N/1000 solution of iodine in aqueous potassium iodide.

The acetyl derivative of the above base, prepared by means of boiling acetic anhydride, crystallised from a mixture of alcohol and light petroleum (b.p. 60-80°) in large, colourless, rectangular plates, m.p. 182-183° (Found: N, 9.6. $C_{27}H_{27}O_2N_3$ requires N, 9.9%). It is insoluble in water. It is readily soluble in ethyl alcohol, methyl alcohol and acetic acid, moderately easily soluble in chloroform and acetone, slightly soluble in ethyl acetate and benzene, and practically insoluble in light petroleum. A dilute alcoholic or acetic acid solution does not give a coloration with an N/1000-solution of iodine in aqueous potassium iodide. The addition of concentrated hydrochloric acid to an alcoholic solution of the acetyl compound gradually precipitates the hydrochloride in rosettes of fine, slender, prismatic needles.

4'-6"-Methoxy-2"-methyl-4"-quinolylamino-3:3'-dimethyl-:diphenylylarsonic Acid (XXVII).

4-o-Tolidino-6-methoxy-2-methylquinoline (7.7 g.) was added to dilute hydrochloric acid (11.4 cc. of hydrochloric acid, d 1.19; 20 cc. of water), and the mixture cooled to 0° during constant stirring. A solution of sodium nitrite (1.6 g.) in water (10 cc.) was then added gradually, care being taken to prevent the temperature rising above 3°. A solid pale orange diazonium salt soon separated, and after all the sodium nitrite had been added, the mixture was allowed to stand for 30 minutes and then cautiously neutralised at 0° with 5N-aqueous sodium hydroxide. Sodium arsenite solution (a mixture of arsenious oxide, 6 g., in 5N-sodium hydroxide solution, 12 cc.; sodium carbonate, 12 g., in water, 36 cc.; and 10% copper sulphate solution, 1.2 cc., to which was added sufficient aqueous ammonium hydroxide to give the soluble complex salt) was then added. Nitrogen was slowly evolved with production of a copious froth, and the mixture was allowed to stand at room-temperature for 18 hours. It was then warmed gently on the water-bath until evolution of nitrogen ceased, filtered, and the solid dark-brown residue extracted thrice with small quantities of hot aqueous sodium hydroxide (5%). The hydrogen-ion/

hydrogen-ion concentration of the combined filtrates was adjusted with hydrochloric acid to pH 7.0; the arsonic acid was then quantitatively precipitated as a light brown gelatinous solid. This was collected, redissolved in warm dilute aqueous sodium hydroxide (animal charcoal), and reprecipitated at the above isolectric point (p_H 7.0) with hydrochloric acid, the process being repeated several times. The acid was finally obtained as a white gelatinous solid. This was filtered off, washed thoroughly with distilled water, and dried at 100°. The acid darkened slightly at 300° and melted at 304-305° (decomp.); yield, 4.4 g. (Found: As, 15.4 $C_{25}H_{25}O_4N_2As$ requires As, 15.2%).

4'-6"-Methoxy-2"-methyl-4"-quinolylamino-3:3'-:dimethyldiphenylylarsonic acid is practically insoluble in water and in the usual neutral organic solvents. It is moderately easily soluble in acetic acid but only slightly soluble in a mixture of alcohol and hydrochloric acid. A saturated solution in hot dilute acetic acid crystallises on cooling in large, colourless, well-shaped, rhombic prisms. The acid dissolves readily in dilute aqueous sodium and ammonium hydroxide solutions, but is only slightly soluble in moderately concentrated hydrochloric acid. A solution in warm concentrated sulphuric acid exhibits a brilliant light blue fluorescence which disappears

on/

on dilution with water. A solution in dilute acetic acid gives a light brown precipitate when added to an N/1000-solution of iodine in aqueous potassium iodide; this gradually changes to dark brown on standing.

The sodium salt is precipitated in a white gelatinous condition when concentrated sodium hydroxide solution is added to a solution of the arsonic acid in dilute alkali; it dissolves on boiling, but is reprecipitated in a gelatinous state on cooling. The magnesium, calcium, barium, silver and mercuric salts are precipitable in a white amorphous condition from an aqueous solution of the ammonium salt: these are practically insoluble in hot water.

4-o-Dianisidino-6-methoxy-2-methylquinoline (XXVIII).

A mixture of 4-bromo-6-methoxy-2-methylquinoline (2.5 g.) and o-dianisidine (7.0 g.) containing a trace of finely divided copper-bronze was heated at 135-140° in an oil-bath during 8 hours. The melt became dark brown and gradually more viscous, and at the end of the period mentioned had almost completely solidified. The reaction-product was digested with boiling dilute hydrochloric acid (5%), and the dirty grey gelatinous hydrochloride, which separated readily on cooling, was filtered off, washed with dilute hydrochloric acid and converted into the crude base (4.1 g.) by warming with aqueous sodium hydroxide solution. On attempting to purify the base by crystallisation from aqueous alcohol (animal charcoal) a considerable quantity of dark brown resinous material separated, and only a small amount (1.3 g.) of fine, pale yellow, rectangular, prismatic needles, m.p. 195-196°, was obtained (Found: C, 72.0; H, 6.1; N, 10.1. $C_{25}H_{25}O_3N_3$ requires C, 72.3; H, 6.1; N, 10.1%) The resinous material which separated during the above crystallisation had phenolic properties and was probably formed by demethylation of the methoxy compounds by the hydrobromic acid liberated.

Attempts/

Attempts to improve the yield of base by carrying out the condensation in either boiling amyl alcohol or nitrobenzene, to which anhydrous potassium carbonate and traces of finely divided copper-bronze and iodine has been added, were entirely unsuccessful, resinous products being obtained in both cases. A very good yield was obtained, however, by carrying out the condensation under diminished pressure as follows. 4-Bromo-6-methoxy-2-methylquinoline (2.5 g.) and o-dianisidine (7.0 g.) were heated together at 135-140° under 10-15 mm. pressure in an oil-bath. The melt rapidly became very viscous with slight frothing and in about 20 minutes had almost completely solidified. The reaction-product was then extracted with boiling dilute hydrochloric acid (5%), and the practically colourless gelatinous hydrochloride, which was readily precipitated on cooling, was filtered off and washed with dilute hydrochloric acid. The pale yellow base, which separated on the addition of ammonium hydroxide solution, was of a high degree of purity and crystallised readily from aqueous alcohol as described above (yield, 4.0 g.).

4-o-Dianisidino-6-methoxy-2-methylquinoline is insoluble in water. It dissolves readily in ethyl alcohol, methyl alcohol, chloroform, acetone, ethyl acetate, and acetic acid. It is moderately easily soluble/

soluble in benzene and ether, but much less soluble in ligroin and light petroleum. A dilute alcoholic solution gives a red coloration with an N/1000-solution of iodine in aqueous potassium iodide; this disappears on warming but does not reappear on cooling. A dilute acetic acid solution, under the same conditions, gradually gives with N/1000-iodine a chocolate coloration, which disappears on warming but reappears on cooling. The base is slightly soluble in boiling dilute hydrochloric, sulphuric and nitric acids, and white gelatinous precipitates of the correponding salts readily separate on cooling. A solution of the diazo-chloride gives a crimson azo-dye on treatment with an alkaline solution of β-naphthol.

The acetyl derivative of the above base crystallises from aqueous alcohol (10% alcohol) in sheaves of fine, colourless, slender needles, m.p. 140° with loss of a molecule of water of crystallisation (Found: C, 68.6; H, 6.1; N, 8.9. $C_{27}H_{27}O_4N_3,H_2O$ requires C, 68.2; H, 6.1; N, 8.8%). After dehydration at 150° the compound has m.p. 200-201°. It is slightly soluble in hot water. It is readily soluble in ethyl alcohol, methyl alcohol, chloroform, acetone, ethyl acetate and acetic acid, moderately easily soluble in benzene, and slightly soluble in ether and light petroleum. The benzene solution exhibits a greenish-blue/

greenish-blue fluorescence. An 0.5% solution in warm dilute acetic acid sets, on cooling, to a firm jelly resembling moderately concentrated gelatine solution. A dilute alcoholic solution gives a brilliant blue coloration with an N/1000-solution of iodine in aqueous potassium iodide: this disappears on warming but does not reappear on cooling. A dilute acetic acid solution, under the same conditions, gives with iodine an intense red coloration which rapidly changes to violet: this practically disappears on warming but reappears on cooling. This violet coloration is given up to a concentration of about N/64,000 iodine.
A violet coloration is also produced when a dilute aqueous solution of potassium iodide is added to a mixture of an acetic acid solution of the acetyl compound and bromine water. The addition of concentrated hydrochloric acid to an alcoholic solution of the acetyl compound slowly precipitates the hydrochloride in sheaves of fine feathery needles.

4'-6"-Methoxy-2"-methyl-4"-quinolylamino-3-3'-dimethoxydiphenylylarsonic Acid (XXIX).

4-o-Dianisidino-6-methoxy-2-methylquinoline (4.2 g.) was added to dilute hydrochloric acid (5.7 cc. of hydrochloric acid, d 1.19; 15 cc. of water) and the mixture cooled to -3° during constant stirring. A solution of sodium nitrite (0.8 g.) in water (8 cc.) was then added very gradually so that the temperature did not rise above 0°. A solid orange diazonium salt separated readily, and after all the sodium nitrite had been added, the mixture was allowed to stand for 30 minutes and then carefully neutralised at -5° with 5N-aqueous sodium hydroxide solution. Sodium arsenite solution (a mixture of arsenious oxide, 3 g., in 5N-sodium hydroxide solution, 6 cc.; sodium carbonate, 12 g., in water, 18 cc.; and 10% copper sulphate solution, 0.6 cc., to which was added sufficient aqueous ammonium hydroxide solution to give the soluble complex salt) was then added. Evolution of nitrogen took place slowly, and the reaction-mixture was allowed to stand overnight at room-temperature. It was warmed gently on the water-bath until effervescence ceased, filtered hot, and the small solid residue of coloured by-products extracted thrice with small quantities of boiling

aqueous/

aqueous sodium hydroxide (5%). The hydrogen-ion concentration of the combined filtrates was adjusted with hydrochloric acid to pH 7.0 and the arsonic acid then completely precipitated in the form of a dirty grey gelatinous solid, was repeatedly dissolved in warm dilute aqueous sodium hydroxide (animal charcoal), and reprecipitated at the above isoelectric point (pH 7.0) with hydrochloric acid. After this treatment, the acid was obtained as a pale grey gelatinous solid. This was filtered off, washed thoroughly with distilled water, and dried at 100°. It darkened slightly at about 220° and melted at 243-245° (decomp.) (Found: As, 14.4. $C_{25}H_{25}O_6N_2As$ requires As, 14.3%); yield of pure material, 3.7 g. (71% of the theoretical).

4'-6"-Methoxy-2"-methyl-4"-quinolylamino-3:3'-dimethoxydiphenylylarsonic acid is practically insoluble in water and in the usual neutral organic solvents. It is moderately easily soluble in acetic acid: a saturated solution in hot dilute acetic acid slowly crystallises on cooling in sheaves of very fine long needles. The acid dissolves readily in dilute aqueous sodium and ammonium hydroxides to give reddish-brown solutions, but it is only slightly soluble in moderately concentrated hydrochloric acid. A solution of the arsonic acid in warm concentrated sulphuric acid exhibits a blue fluorescence which disappears on dilution/

dilution with water. A dilute acetic acid solution slowly gives a dark brown flocculent precipitate when added to an N/1000-solution of iodine in aqueous potassium iodide.

The sodium salt is precipitated as a light grey gelatinous mass when concentrated sodium hydroxide solution is added to a solution of the arsonic acid in dilute alkali. The magnesium, calcium, and barium salts are white and gelatinous, and the silver and mercuric salts are pale yellow and curdy; all are insoluble in water.

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p-6-Methoxy-2-methyl-4-quinolylamino-p'-aminodiphenylmethane (XXIV).

A mixture of pp'-diaminodiphenylmethane (6 g.) and 4-bromo-6-methoxy-2-methylquinoline (2.5 g.) was heated at 130-135° under a pressure of 10-15 mm. The melt rapidly became very viscous with slight frothing and in about 30 minutes was almost completely converted into a light brown crystalline solid. This was extracted with a slight excess of boiling dilute hydrochloric acid (5%), and the pale greenish yellow gelatinous hydrochloride, which readily separated on cooling, was filtered off, washed with dilute hydrochloric acid, and converted into the free base by warming with ammonium hydroxide solution (yield, 3.7 g.). It crystallised from aqueous alcohol (20% alcohol) in pale yellow rectangular prismatic needles (radially arranged), m.p. 135-145° with loss of a molecule of water of crystallisation (Found: C, 74.1; H, 6.7; N, 10.6. $C_{24}H_{23}ON_3,H_2O$ requires C, 74.4; H, 6.5; N, 10.8%).

p-6-Methoxy-2-methyl-4-quinolylamino-p'-aminodiphenylmethane is insoluble in water. It is readily soluble in ethyl alcohol, methyl alcohol, chloroform, acetone, ethyl acetate and acetic acid, slightly soluble/

soluble in ether and benzene, and practically insoluble in light petroleum. A dilute alcoholic or acetic acid solution does not give a coloration with an N/1000-solution of iodine in aqueous potassium iodide. This is in sharp contrast to the behaviour of the corresponding diquinolyl derivative, which gives with iodine a deep purple coloration (see p. 126). A dilute alcoholic or acetic acid solution containing both the mono- and the di-quinolyl compound also gives a deep purple coloration on the addition of iodine even when the former is present in large excess, and the non-production of a colour, under these conditions, with the monoquinolyl derivative alone indicates that it is free from contamination with the diquinolyl compound. The base is slightly soluble in boiling dilute hydrochloric, sulphuric and nitric acids and, on cooling, crystalline precipitates of the corresponding salts separate. A solution of the diazo-chloride readily gives a scarlet azo-dye on treatment with an alkaline solution of β-naphthol.

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p-6-Methoxy-2-methyl-4-quinolylaminodiphenylmethane-p'-arsonic Acid (XXXI).

p-6-Methoxy-2-methyl-4-quinolylamino-p'-amino-diphenylmethane (3.7 g.) was added to dilute hydrochloric acid (5.7 cc. of hydrochloric acid, d 1.19; 15 cc. of water), and the mixture cooled during constant stirring to 15° (diazotisation does not take place below this temperature). A solution of sodium nitrite (0.8 g.) in water (5 cc.) was then added gradually care being taken to prevent the temperature rising above 20°. After all the sodium nitrite had been added the mixture was allowed to stand for 30 minutes and then cautiously neutralised at 0° with 5N-aqueous sodium hydroxide. Sodium arsenite solution (a mixture of arsenious oxide, 3 g., in 5N sodium hydroxide solution, 6 cc., sodium carbonate, 6 g., in water, 18 cc.; and 10% copper sulphate solution, 0.6 cc., to which was added sufficient aqueous ammonium hydroxide to give the soluble complex salt) was then added. A vigorous evolution of nitrogen took place and the mixture was allowed to stand at room-temperature overnight. It was then warmed gently on the water-bath, filtered, and the bulky solid brown residue of by-product extracted thrice with small amounts of boiling aqueous sodium hydroxide solution/

solution (5%). The hydrogen-ion concentration of the combined filtrates was adjusted with hydrochloric acid to pH 6.5; the arsonic acid was then completely precipitated as a pale yellow gelatinous solid. This was collected, redissolved in warm dilute sodium hydroxide solution, and reprecipitated at the above isoelectric point (pH 6.5) with hydrochloric acid. It was then filtered off, washed with distilled water and dried at 100°. It darkened slightly at 280° and charred at about 300° (yield, 1.1 g.) (Found: As, 16.0. $C_{24}H_{23}O_4N_2As$ requires As, 15.7%).

p-6-Methoxy-2-methyl-4-quinolylaminodiphenylmethane-p'-arsonic acid is practically insoluble in water and in the usual neutral organic solvents. It is readily soluble in acetic acid. It dissolves readily in dilute aqueous sodium and ammonium hydroxide solutions, but is only slightly soluble in moderately concentrated hydrochloric acid. A solution of the arsonic acid in concentrated sulphuric acid exhibits a brilliant purplish-blue fluorescence which is destroyed on dilution with water. A dilute acetic acid solution gives a light brown amorphous precipitate with an N/1000-solution of iodine in aqueous potassium iodide: this dissolves on warming but is reprecipitated on cooling. The sodium salt is precipitated in a white gelatinous state when concentrated sodium hydroxide solution is added to a solution of the arsonic acid in dilute alkali.

4-Anilino-6-methoxy-2-methylquinoline (XXXII).

A mixture of 4-chloro-6-methoxy-2-methylquinoline (5.2 g.) and aniline (10 g.) was heated along with a trace of finely divided copper-bronze at 180° during 5 hours. The light brown mixture was rendered alkaline with aqueous sodium hydroxide solution, and then submitted to steam-distillation in order to remove the excess of unchanged aniline. The residue, which solidified, was dissolved in absolute alcohol, and the solution saturated with dry hydrogen chloride; the monohydrochloride of the base then readily separated in fine, pale yellow, sharp-pointed, rectangular, prismatic needles which, on heating, darkened slightly at 300° but were unmolten at 310°; yield, 6.0 g. (Found: C, 67.6; H, 6.0; N, 9.3. $C_{17}H_{16}ON_2$,HCl requires C, 67.9; H, 5.7; N, 9.3%). The free base was liberated by warm aqueous sodium hydroxide, and crystallised from benzene, containing a few chips of potassium hydroxide, in tufts of fine, slender, colourless needles, m.p. 208-209°.

4-Anilino-6-methoxy-2-methylquinoline is insoluble in water. It is readily soluble in ethyl alcohol, methyl alcohol, chloroform, acetone, ethyl acetate, ether, and acetic acid, moderately easily soluble in benzene and ligroin, and slightly soluble in/

in light petroleum. A dilute alcoholic or acetic acid solution does not give any coloration with an N/1000-solution of iodine in aqueous potassium iodide. The base is slightly soluble in boiling dilute hydrochloric, sulphuric and nitric acids and, on cooling, crystalline precipitates of the corresponding salts separate.

8-Bromo-5-nitroquinoline (XXXIII).

To a solution of 8-aminoquinoline (30 g.) in hydrobromic acid (150 cc. of hydrobromic acid, d 1.49; 300 cc. of water), crushed ice (400 g.) was added, and the cold stirred mixture diazotised with a solution of sodium nitrite (15 g.) in water (30 cc.). The diazo-solution was added gradually to a solution of cuprous bromide (40 g.; precipitated from copper sulphate-potassium bromide solution by sulphur dioxide) in hydrobromic acid (400 cc.; d 1.49) at 60-70°. After standing overnight at room-temperature the orange-red crystalline precipitate of the cuprous bromide salt of 8-bromoquinoline was filtered off, washed with water, added in small portions at a time to well-stirred sodium hydroxide solution (100 cc. of 50%), and the liberated base extracted several times with ether. To the oil obtained after removal of the ether, nitric acid (50 cc.; d 1.5) was carefully added so that the temperature did not rise above 80-90°, and then concentrated sulphuric acid (50 cc.) was poured in. The reaction-mixture was warmed on the water-bath for 1 hour to complete the nitration, cooled, and added to water (4 litres); the copious, pale yellow precipitate of pure 8-bromo-5-nitroquinoline was filtered off, washed with water, and dried at 100°; m.p. 136-137°; yield, 36-38 g. (compare Dikshoorn, Rec. trav. chim., 1929, 48, 550).

Condensation of 8-Bromo-5-nitroquinoline with o-Amino-phenylarsonic Acid.

8-Bromo-5-nitroquinoline (8.4 g.) and o-amino-phenylarsonic acid (7.3 g.) were dissolved in dry amyl alcohol (50 cc.) and anhydrous potassium carbonate (6.3 g.) and traces of finely divided copper-bronze and iodine added. The mixture was kept at 140-150° for 8 hours, cooled, and the amyl alcohol removed by distillation in steam. The residual dark reddish-brown solution was filtered and the solid residue (A) extracted several times with boiling dilute sodium carbonate solution. The hydrogen-ion concentration of the combined filtrates was adjusted with hydrochloric acid to pH 3-4; the small amount (about 1 g.) of dark reddish-brown solid thereby precipitated was difficult to purify, and after several reprecipitations from solution in dilute aqueous sodium carbonate (animal charcoal) at pH 3-4 it was still highly coloured. It had m.p. about 200°, and on analysis was found to contain As, 13.4% (o-5'-nitro-8'-quinolylaminophenylarsonic acid (XXXIV) requires As, 19.3%). The residue (A) was partly soluble in dilute sodium hydroxide solution (the insoluble portion was found to consist chiefly of unchanged 8-bromo-5-nitroquinoline), and on acidification of the/

the red alkaline filtrate with hydrochloric acid to pH 3.5-4.5 a pale yellow product (2.5 g.) was precipitated. This compound had m.p. 172-173° and was arsenic-free. It was shown to be identical with 5-nitro-8-hydroxyquinoline. The same products were obtained in approximately the same proportions when the reaction was carried out in the absence of traces of copper-bronze and iodine.

5-Nitro-8-aminoquinoline (XXXV).

A mixture of 8-bromo-5-nitroquinoline (14 g.) and saturated methyl alcoholic ammonia (100 cc.) was heated in a sealed tube at 140° for 4 hours. After cooling, the contents of the tube were added to excess of water, and the orange-red precipitate filtered off and dried at 100°. The base crystallised from benzene in fine orange needles, m.p. 196-197° (compare Dikshoorn, Rec. trav. chim., 1929, 48, 520); yield, 9.2 g.

The acetyl derivative of the above base, prepared by means of boiling acetic anhydride in the usual way, crystallised from light petroleum (b.p. 60-80°) in slender, bright yellow, rectangular prismatic needles, m.p. 167-168°, (Found: C, 57.3; H, 3.9; N, 18.1. $C_{11}H_9O_3N_3$ requires C, 57.1; H, 3.9; N, 18.2%). It is practically insoluble in water. It is readily soluble in chloroform, moderately easily soluble in alcohol, benzene, and acetic acid, but much less soluble in light petroleum. It dissolves readily in hydrochloric, sulphuric and nitric acids. A dilute acetic acid solution of the acetyl derivative gives a transient deep greenish-blue coloration with an N/1000-solution of iodine in aqueous potassium iodide.

o-5'Nitro-8'-quinolylaminophenylarsonic Acid (XXXIV).

5-Nitro-8-aminoquinoline (6.3 g.) and o-bromophenylarsonic acid (9.4 g.) were dissolved in dry amyl alcohol (60 cc.) and anhydrous potassium carbonate (6.3 g.) and traces of finely divided copper-bronze and iodine added. The mixture was kept at 140-150° for 10 hours, cooled, and the amyl alcohol removed by distillation in steam. The residual dark reddish-brown solution was filtered and the solid residue (A) extracted several times with small amounts of boiling dilute sodium carbonate solution. The hydrogen-ion concentration of the combined filtrates was adjusted with hydrochloric acid to pH 3-4; the bright yellow arsonic acid thereby precipitated recrystallised from acetic acid (70%) in stellate clusters of fine flat yellow needles, m.p. 264-265° (decomp.); yield, 3.2 g. (Found: As, 19.5. $C_{15}H_{12}O_5N_3As$ requires As, 19.3%). The solid residue (A) and the solid precipitated by water from the mother liquors from the above crystallisation consisted chiefly of unchanged 5-nitro-8-aminoquinoline and o-bromophenylarsonic acid respectively.

o-5'-Nitro-8'-quinolylaminophenylarsonic acid is practically insoluble in water and in the usual neutral organic solvents, but it is readily soluble in a mixture/

mixture of alcohol and hydrochloric acid and moderately easily soluble in acetic acid. It dissolves readily in dilute sodium and ammonium hydroxides to give orange coloured solutions and in hydrochloric, sulphuric and nitric acids to give yellow solutions: the arsonic acid is reprecipitated from the acid solutions on dilution with water. An acetic acid solution of the arsonic acid gives a brown precipitate with N/1000-iodine. The arsonic acid is not converted into the arsazinic acid (XLIII) by hydrochloric acid even on prolonged boiling (compare Gibson and Johnson, J., 1927, 2501).

The sodium salt is very slowly precipitated in orange rectangular plates when concentrated sodium hydroxide solution is added to a solution of the arsonic acid in dilute alkali. The ammonium salt is slowly precipitated in sheaves of fine, orange, sharp-pointed needles in a similar way. The following salts are precipitable from an aqueous solution of the ammonium salt: calcium salt, bright yellow, gelatinous, insoluble in hot water; barium salt, bright yellow, crystalline, slightly soluble in hot water, sheaves of fine needles on cooling. The addition of magnesium sulphate solution causes the orange solution of the ammonium salt of the arsonic acid to become orange-red but no precipitate is formed in the cold: the magnesium salt is however precipitated, on boiling, in an orange gelatinous/

gelatinous condition. The following salts are precipitated in an amorphous condition and are insoluble in hot water: zinc salt, reddish-brown; silver salt, blood red, soluble in ammonium hydroxide to give an orange solution; lead and mercuric salts, bright red; ferric salt, dark brown; copper, nickel and cobalt salts, vermilion.

12-Chloro-7-methoxy-11-methyl-5:12-dihydroquinbenzarsazine (XXXIX).

o-6'-Methoxy-2'methyl-4'-quinolylaminophenylarsonic acid (1.9 g.) was dissolved in a mixture of absolute alcohol (15 cc.) and hydrochloric acid (10 cc; d 1.19), and a small crystal of iodine added. The clear brown solution was heated to its boiling-point and a current of sulphur dioxide bubbled through. Reduction and ring-closure took place rapidly, and in a few minutes a copious light brown crystalline mass was precipitated. On cooling, this was filtered off and dried (1.7 g.). It was purified by re-crystallisation from hot water and separated in small, pale yellow, rhombohedral plates, which darkened slightly at 235° and melted at 245-247° (decomp.) (Found: Cl, 9.0; As, 20.2. $C_{17}H_{14}ON_2ClAs$ requires Cl, 9.5; As, 20.1%).

12-Chloro-7-methoxy-11-methyl-5:12-dihydroquinbenzarsazine is moderately easily soluble in hot water, but it is practically insoluble in the usual neutral organic solvents. It dissolves readily in acetic acid. It is slightly soluble in hot dilute sodium hydroxide solution and in boiling hydrochloric acid; these/

these solutions deposit stillate clusters of fine, rectangular, prismatic needles on cooling. A dilute acetic acid solution does not give a coloration with an N/1000-solution of iodine in aqueous potassium iodide.

7-Methoxy-11-methylquinbenzarsazinic Acid (XL).

12-Chloro-7-methoxy-11-methyl-5:12-dihydroquinbenzarsazine (0.9 g.) was dissolved in hot acetic acid (5 cc.) and cooled, and hydrogen peroxide (10 cc; 10 vols.) added. The pale yellow solution rapidly turned reddish-brown and exhibited a brilliant blue fluorescence. The mixture was allowed to stand at room-temperature for 5 minutes and then warmed on the water-bath to complete the oxidation. The hydrogen-ion concentration of the solution was adjusted with aqueous sodium hydroxide to pH 7.0; the arsazinic acid was then quantitatively precipitated as a pale yellow gelatinous solid. This was collected, dissolved in dilute aqueous sodium hydroxide solution, and reprecipitated at its isoelectric point (pH 7.0) with hydrochloric acid. It was then filtered off, washed with distilled water and dried at 100° (yield, 0.7 g.). The acid was unmolten at 310° (turning slightly brown) (Found: As, 20.5. $C_{17}H_{15}O_3N_2As$ requires As, 20.3%).

7-Methoxy-11-methylquinbenzarsazinic acid is practically insoluble in water and in the usual neutral organic solvents. It dissolves readily in acetic acid, the solution exhibiting an intense blue fluorescence. It is soluble in dilute aqueous sodium and ammonium/

ammonium hydroxide solutions and in hot concentrated hydrochloric acid: these solutions also exhibit a blue fluorescence. The hydrochloric acid solution deposits small prismatic needles on cooling.
A solution of the arsazinic acid in warm concentrated sulphuric acid exhibits a brilliant blue fluorescence, which is not destroyed on dilution with water.
A dilute acetic acid solution does not give a coloration with an N/1000-solution of iodine in aqueous potassium iodide.

The sodium salt is precipitated in a white gelatinous state when concentrated sodium hydroxide solution is added to a solution of the arsazinic acid in dilute alkali: this dissolves on heating and crystallises on cooling in fine colourless, sharp-pointed, prismatic needles. The following salts are precipitable from an aqueous solution of the ammonium salt; magnesium salt, sheaves of fine long colourless needles, soluble in hot water; barium salt, white, gelatinous, soluble in hot water, crystallises on cooling in stellate clusters of fine prismatic needles; calcium salt, white, gelatinous insoluble in hot water; silver salt, white, gelatinous, insoluble in hot water; mercuric salt, white, curdy, insoluble in hot water.

7-Methoxy-11-methylquinbenzarsazinyl Chloride

(XLI).

o-6'-Methoxy-2'-methyl-4'-quinolylaminophenylarsonic acid (1.9 g.) was added gradually to phosphoryl chloride (8 cc.) care being taken to ensure that the temperature did not rise above 80°. The vigorous reaction, which took place, rapidly subsided, and the reaction-mixture was warmed on the water-bath until evolution of hydrogen chloride ceased (about 15 minutes). The excess of phosphoryl chloride was then cautiously decomposed with water, and the bright yellow solid which separated was dissolved in the minimum amount of warm dilute acetic acid (10%). The solution, after filtration, was neutralised with dilute aqueous sodium hydroxide; the arsazinyl chloride was then completely precipitated as a pale yellow, gelatinous solid. This was collected, washed thoroughly with distilled water, and dried (yield, 1.4 g.). It darkened slightly at 150° and melted at 165-167° (Found: Cl, 8.9; As, 19.6. $C_{17}H_{14}O_2N_2ClAs$ requires Cl, 9.1; As, 19.3%).

7-Methoxy-11-methylquinbenzarsazinyl chloride is insoluble in water. It is readily soluble in acetic acid, slightly soluble in acetone and in boiling benzene,/

benzene, and practically insoluble in ligroin and light petroleum. It slowly dissolves in boiling dilute aqueous sodium hydroxide, the solution exhibiting a bright blue fluorescence. It is readily soluble in boiling dilute hydrochloric acid. A dilute acetic acid solution does not give a coloration with an N/1000-solution of iodine in aqueous potassium iodide.

12-Chloro-10-nitro-5:12-dihydroquinbenzarsazine Hydrochloride (XLII).

o-5'-Nitro-8'-quinolylaminophenylarsonic acid (2.5 g.) was dissolved in a mixture of alcohol (20 cc.) and hydrochloric acid (15 cc.; d 1.19), and a small crystal of iodine added. The clear reddish-brown solution was gently boiled and sulphur dioxide bubbled through. In a few minutes the quinbenzarsazine hydrochloride crystallised out in stellate clusters of dark red feathery needles which turned yellowish-brown at about 200° and melted at 258-260° (decomp.) (Found: N, 9.9; Cl, 16.8; As, 18.5. $C_{15}H_{10}O_2N_3Cl_2As$ requires N, 10.2; Cl, 17.3; As, 18.3%). Yield: 2.4 g.

The chlorine atoms in this chlorodihydroquinbenzarsazine hydrochloride are readily split off on warming with 4 molecules of N/10-sodium hydroxide solution or on boiling with water for a few minutes with production of an orange compound, m.p. 275-277° (decomp.) which is presumably 10-nitro-12-hydroxy-5:12-dihydroquinbenzarsazine. (XLIV). This orange compound is readily reconverted into 12-chloro-10-nitro-5:12-dihydroquinbenzarsazine hydrochloride on boiling with hydrochloric acid, and on warming with acetic acid containing hydrogen peroxide it is oxidised to 10-nitroquinbenzarsazinic acid. (XLIII).

10-Nitroquinbenzarsazinic Acid (XLIII).

Finely powdered 12-chloro-10-nitro-5:12-dihydroquinbenzarsazine hydrochloride (2 g.) was boiled in acetic acid (10 cc.) for a few minutes, cooled rapidly, and hydrogen peroxide (20 cc.; 10 vols) added. No reaction appeared to take place in the cold, but when warmed the fine red suspension was gradually converted into a bright yellow compound, and the mixture was kept on the water-bath for 15 minutes to complete the oxidation. When cold the yellow solid was filtered off (1.7 g.): it crystallised from much acetic acid in clusters of fine, small, yellow needles which darkened slightly at about 300° but were unmolten at 310° (Found: As, 20.4. $C_{15}H_{10}O_4N_3As$ requires As, 20.2%).

10-Nitroquinbenzarsazinic acid is practically insoluble in water and in the usual neutral organic solvents, but it is slightly soluble in acetic acid. It dissolves readily in dilute potassium hydroxide solution to give a bright yellow solution which changes to red and then to a brilliant purple on the addition of concentrated aqueous potassium hydroxide. The original yellow solution reappears on dilution with water and can be reconverted into the purple solution on the addition of concentrated alkali. A solution of the arsazinic acid in dilute aqueous sodium hydroxide behaves similarly. The arsazinic acid dissolves/

dissolves in dilute ammonium hydroxide solution to give an orange solution which changes to bright red on the addition of excess of concentrated ammonium hydroxide. An acetic acid solution of the arsazinic acid gives a dark brown precipitate with N/1000-iodine. The arsazinic acid is readily soluble in sulphuric and nitric acids (yellow solutions), but is reprecipitated on dilution with water. It is slightly soluble in hydrochloric acid. On reduction in boiling alcohol-hydrochloric acid solution with sulphur dioxide it is converted into the dark red chlorodihydroquinbenzarsazine hydrochloride (XLII).

The potassium salt is precipitated in a purple gelatinous state when concentrated potassium hydroxide is added to a solution of the arsazinic acid in dilute alkali. The purple sodium salt and the orange ammonium salt can be precipitated (the latter very slowly) in the same way. The following salts are precipitable from an aqueous solution of the ammonium salt: magnesium salt, yellow, gelatinous, slightly soluble in hot water, crystallises on cooling in long sharp-pointed needles; the barium and calcium salts are yellow, gelatinous, soluble in hot water, and crystallise on cooling in sheaves of rectangular prismatic needles and clusters of fine small needles respectively. The following are insoluble in hot water:/

water: silver salt, violet, amorphous, soluble in ammonium hydroxide to give a red solution: mercuric salt, bright red, amorphous; lead salt, yellow gelatinous, changes to red in boiling water and remains red on cooling; cupric salt, deep brick red, amorphous; ferric salt, orange, gelatinous; zinc salt, red, gelatinous; nickel salt, brown, gelatinous; cobalt salt, reddish-brown, gelatinous.

8-Piperidino-5-nitroquinoline (LI).

A mixture of 8-bromo-5-nitroquinoline (7.5 g.) and piperidine (15 g.) was gently heated on the water-bath to 80°. The vigorous reaction, which took place at this temperature, rapidly subsided and the solution was heated on the boiling water-bath for 30 minutes in order to complete the reaction. The product was then poured into excess of water and the orange yellow oil, which was precipitated, solidified in a few minutes. The base was purified by recrystallisation from alcohol and separated in fine, long, yellow, rectangular prismatic needles, m.p. 95-96°; yield, 7.1 g. (Found: C, 65.4; H, 5.7; N, 16.1. $C_{14}H_{15}O_2N_3$ requires C, 65.4; H, 5.9; N, 16.3%).

8-Piperidino-5-nitroquinoline is practically insoluble in water. It is readily soluble in chloroform and acetic acid, moderately easily soluble in alcohol and benzene, but only slightly soluble in light petroleum. It dissolves readily in hydrochloric and nitric acids to give yellow solutions. Its solution in sulphuric acid has a pale pink colour but this becomes yellow on dilution with water. A dilute alcoholic or acetic acid solution of the base does not give a coloration with N/1000-iodine.

8-Piperidino-5-aminoquinoline.

Iron filings (17 g.) were added in small portions to a constantly boiling solution of 8-piperidino-5-nitroquinoline (26 g.) in alcohol (200 cc.) containing hydrochloric acid (25 cc.; d 1.19), and the mixture boiled for 2 hours. Sodium ethoxide (6 g. of sodium in 150 cc. of alcohol) was then added and the whole refluxed for a few minutes. The mixture of iron and ferric hydroxide was filtered off and extracted thrice with small quantities of boiling alcohol. The combined filtrates were then acidified with hydrochloric acid and submitted to steam-distillation to remove the alcohol. The residual reddish-brown hydrochloric acid solution was filtered, and rendered alkaline with ammonium hydroxide solution. The brown flocculent base thus liberated crystallised from a mixture of benzene and light petroleum (b.p. 60-80°) in stellate clusters of fine, long, yellow, silky needles, m.p. 182-183° (Found: C, 73.8; H, 7.5; N, 18.1. $C_{14}H_{17}N_3$ requires C, 74.0; H, 7.5; N, 18.5%). Yield, 12 g.

8-Piperidino-5-aminoquinoline is practically insoluble in water. It is readily soluble in alcohol, benzene, chloroform and acetic acid, moderately easily soluble in ligroin, but only slightly soluble in light petroleum/

petroleum. It dissolves readily in hydrochloric and nitric acids to give yellow solutions. Its solution in sulphuric acid has a pale pink colour which becomes yellow on dilution. A dilute alcoholic solution of the base gives a red coloration with N/1000-iodine: this changes to green on warming. A solution of the diazo-chloride gives a deep purple azo-dye on treatment with an alkaline solution of β-naphthol.

The acetyl derivative of the above base, prepared by means of boiling acetic anhydride, crystallised from benzene in fine, long, pale yellow, prismatic needles, m.p. 210-211° (Found: C, 71.2; H, 7.2; N, 15.5. $C_{16}H_{19}ON_3$ requires C, 71.3; H, 7.1; N, 15.6%). It is practically insoluble in water. It is readily soluble in alcohol, chloroform and acetic acid, moderately easily soluble in benzene, but sparingly soluble in light petroleum. It dissolves readily in hydrochloric, sulphuric and nitric acids. A dilute alcoholic or acetic acid solution of the acetyl compound does not give a coloration with N/1000-iodine.

8-Bromo-5-aminoquinoline (LIII).

8-Bromo-5-nitroquinoline (25.3 g.) was reduced in boiling alcohol-hydrochloric acid solution (150 cc. of alcohol/

alcohol and 15 cc. of acid of d 1.19) by means of iron filings (17 g.) under the same conditions used in the preparation of 8-piperidino-5-aminoquinoline (see p. 169). The base was liberated from its hydrochloric acid solution in a crystalline condition by means of excess of concentrated aqueous sodium hydroxide (ammonium hydroxide does not effect complete precipitation), and purified by recrystallisation from aqueous alcohol (20% alcohol). It separated in brownish-yellow, feathery needles, m.p. 156-157°; yield, 19 g. (Found: C, 48.3; H, 2.9; N, 12.2. $C_9H_7N_2Br$ requires C, 48.4; H, 3.2; N, 12.6%).

8-Bromo-5-aminoquinoline is practically insoluble in water. It is readily soluble in alcohol, chloroform and acetic acid, moderately easily soluble in benzene, but only sparingly soluble in light petroleum. Its acetic acid solution has an intense red colour. It dissolves in hydrochloric, nitric and sulphuric acids to give yellow solutions which become intense orange-red on dilution with water. A solution of the diazo-chloride gives a crimson azo-dye on treatment with an alkaline solution of β-naphthol. A dilute alcoholic or acetic acid solution of the base does not give a coloration with N/1000-iodine.

The acetyl derivative of the above base, prepared by means of boiling acetic anhydride, crystallised from benzene in sheaves of light brown, rectangular prismatic/

prismatic needles, m.p. 179-180° (Found: C, 49.8; H, 3.5; N, 10.3. $C_{11}H_9ON_2Br$ requires C, 49.8; H, 3.4; N, 10.6%). It is readily soluble in alcohol, chloroform and acetic acid, slightly soluble in water and benzene, but practically insoluble in light petroleum. It dissolves readily in hydrochloric, sulphuric and nitric acids. A dilute alcoholic or acetic acid solution of the acetyl compound does not give a coloration with N/1000-iodine.

8-Bromoquinoline-5-arsonic Acid. (LIV).

8-Bromo-5-aminoquinoline (5.6 g.) was dissolved in dilute hydrochloric acid (12 cc. of d 1.19 acid; 50 cc. of water), and the solution cooled to 0° during constant stirring. A solution of sodium nitrite (2 g.) in water (5 cc.) was then added gradually so that the temperature did not rise above 5°. The mixture was allowed to stand for 1 hour and then neutralised at 0° with aqueous sodium hydroxide (5N). Sodium arsenite solution (a mixture of arsenious oxide, 4 g., in 5N-sodium hydroxide solution, 8 cc.; sodium carbonate, 8 g., in water, 24 cc.; and 10% copper sulphate solution, 0.8 cc., to which was added sufficient aqueous ammonium hydroxide to give the soluble/

soluble complex salt) was then added. Nitrogen was slowly evolved and the mixture was kept at room-temperature overnight. It was then warmed gently on the water-bath until evolution of nitrogen ceased, filtered, and the bulky solid residue extracted thrice with small amounts of aqueous sodium hydroxide (5%). The reaction of the combined filtrates was adjusted with hydrochloric acid to pH 3-4; the arsonic acid was then precipitated in sheaves of light brown needles. These were redissolved in hot aqueous sodium carbonate solution (animal charcoal) and reprecipitated at the above isoelectric point (pH 3-4) with hydrochloric acid. The arsonic acid crystallised from dilute acetic acid in stellate clusters of flat colourless needles, m.p. 234-235° (decomp.); yield, 0.8 g. (Found: N, 4.3; Br, 23.8; As, 22.4. $C_9H_7O_3NBrAs$ requires N, 4.2; Br, 24.1; As, 22.6%).

The yield of 8-bromoquinoline-5-arsonic acid was greatly improved by carrying out the Bart reaction in acid solution as follows.

A solution of 8-bromo-5-aminoquinoline (5.6 g.) in dilute hydrochloric acid (30 cc. of d 1.12 acid and 12 cc. of water) was diazotised at 0° with a solution of sodium nitrite (2 g.) in water (5 cc.). The diazo-mixture was allowed to stand for 1 hour and then poured into a solution of sodium arsenite (10.5 g.) in water

(25/

(25 cc.) to which saturated copper sulphate solution (2.5 cc.) had been added. The mixture was kept at room-temperature overnight and then warmed on the water-bath until evolution of nitrogen ceased. It was then adjusted with sodium hydroxide solution (30%) to pH 3-4, and the arsonic acid thereby quantitatively precipitated was collected and purified as described above; yield of pure material, 5.3 g. (63.6 per cent of the theoretical).

8-Bromoquinoline-5-arsonic acid is slightly soluble in hot water. It is readily soluble in acetic acid, moderately easily soluble in alcohol, but practically insoluble in benzene, chloroform and light petroleum. The arsonic acid dissolves readily in dilute aqueous sodium and ammonium hydroxides and in concentrated hydrochloric and sulphuric acids. A dilute alcoholic or acetic acid solution of the arsonic acid does not give a coloration with N/1000-iodine.

The white gelatinous sodium salt is readily produced when concentrated sodium hydroxide solution is added to a solution of the arsonic acid in dilute alkali; it dissolves on boiling and crystallises on cooling in fine slender needles. The following salts are precipitable from an aqueous solution of the ammonium salt: silver salt, white, gelatinous; lead/

lead salt, white, gelatinous; mercuric salt, white, gelatinous; cupric salt, greenish-blue, gelatinous; all are insoluble in hot water. The calcium salt is not precipitated in the cold but readily separates in a white gelatinous condition on boiling; it redissolves on cooling. The barium and magnesium salts appear to be readily soluble and are not precipitated in the usual way.

8-Chloro-5-aminoquinoline.

8-Chloro-5-nitroquinoline (16 g.; prepared from 8-chloroquinoline as described by Fourneau, Tréfouel and Wancolle, Bull. Soc. chim., 1930, 47, 740) was reduced in alcohol-hydrochloric acid solution by means of iron filings under exactly the same conditions used in the preparation of 8-bromo-5-aminoquinoline (see p. 170). The base (12 g.) liberated from the hydrochloric acid solution by means of excess of concentrated aqueous sodium hydroxide crystallised from aqueous alcohol (10% alcohol) in fine, long, light brown, slender needles, m.p. 154-155° (Found: C, 60.6; H, 4.1; N, 15.4. $C_9H_7N_2Cl$ requires C, 60.5; H, 4.0; N, 15.7%). Claus and Schöller (J. pr. Chem., 1893, 48, 146/

146) give m.p. 152°.

8-Chloro-5-aminoquinoline resembles 8-bromo-5-aminoquinoline very closely in its properties.

The acetyl derivative of the above base, prepared by means of acetic anhydride, crystallised from hot water in sheaves of fine, colourless, slender needles, which contained solvent of crystallisation. After being heated for 3 hours at 100°, it had m.p. 172-173° (Found: C, 59.8; H, 4.1; N, 12.6. $C_{11}H_9ON_2Cl$ requires C, 59.9; H, 4.1; N, 12.7%). Except for being readily soluble in hot water, this acetyl derivative closely resembles its bromo-analogue (see p. 171).

8-Chloroquinoline-5-arsonic Acid (LVI).

This arsonic acid was prepared from 8-chloro-5-aminoquinoline (4.5 g.) in exactly the same way as in the second method of preparation of 8-bromoquinoline-5-arsonic acid (see p. 173). The chloroquinolylarsonic acid (4.1 g.) was precipitated in a crystalline condition from its solution in aqueous sodium carbonate on acidification with hydrochloric acid to pH 3-4. It recrystallised from dilute acetic acid in sheaves of fine, slender, colourless needles, m.p. 226-227° (decomp.)/

(decomp.) (Found: As, 26.3. $C_9H_7O_3NClAs$ requires As, 26.1%).

8-Chloroquinoline-5-arsonic acid resembles 8-bromoquinoline-5-arsonic acid very closely in its properties.

5-Chloroquinoline-8-arsonic Acid (LVII).

Finely powdered 5-nitro-8-aminoquinoline (5.5 g.) was added to hydrochloric acid (55 cc. of d 1.12 acid and 16 cc. of water), and the mixture diazotised at 15° with a solution of sodium nitrite (2.5 g.) in water (7 cc.) (diazotisation does not appear to take place readily below 15°). The diazo-mixture was allowed to stand for 2 hours and then poured into a solution of sodium arsenite (21 g.) in water (50 cc.) to which saturated copper sulphate solution (5 cc.) had been added. No reaction took place until the mixture was heated to 90-100°; nitrogen was then slowly evolved and the mixture was kept at this temperature for 1 hour. During this time small quantities of sodium nitrite solution (40%) were constantly added to ensure that there was always a slight excess of nitrous acid present. The mixture was then made slightly alkaline by the addition of sodium/

sodium hydroxide solution, and filtered, and the residue extracted thrice with small amounts of boiling aqueous sodium hydroxide (5%). The reaction of the combined filtrates was adjusted with hydrochloric acid to pH 3-4; the arsonic acid was then quantitatively precipitated in the form of yellow needles, which were collected, redissolved in warm sodium carbonate solution (animal charcoal), and reprecipitated at the above isoelectric point (pH 3-4) with hydrochloric acid. The arsonic acid crystallised from dilute acetic acid in fine, slender, colourless needles, m.p. 284-285° (decomp.) (Found: N, 4.7; Cl, 12.2; As, 26.4. $C_9H_7O_3NClAs$ requires N, 4.9; Cl, 12.3; As, 26.1%). Yield, 4.9 g. (58.6 per cent. of the theoretical).

5-Chloroquinoline-8-arsonic acid is slightly soluble in water. It is readily soluble in acetic acid, moderately easily soluble in alcohol, sparingly soluble in benzene, but practically insoluble in chloroform and light petroleum. The arsonic acid dissolves readily in dilute aqueous sodium and ammonium hydroxides and in concentrated hydrochloric and sulphuric acids. A dilute alcoholic or acetic acid solution does not give a coloration with N/1000-iodine.

The pale yellow gelatinous sodium salt is readily produced when concentrated sodium hydroxide solution is added to a solution of the arsonic acid in dilute alkali;/

alkali; it dissolves on boiling and sets to a firm gel on cooling. The following salts are precipitable from an aqueous solution of the ammonium salt: calcium salt, white, gelatinous; silver salt, white, gelatinous; lead salt, white, gelatinous; mercuric salt, pale yellow, gelatinous; cupric salt, green, gelatinous: all are insoluble in hot water. The barium and magnesium salts are not precipitated in the cold but readily separate in stellate clusters of colourless needles and in a white gelatinous condition, respectively, on boiling: these precipitates do not redissolve on cooling (compare behaviour of the calcium salt of 8-bromoquinoline-5-arsonic acid, p. 175).

5-Chloro-6-nitroquinoline-8-arsonic Acid (LVIII).

To a solution of 5-chloroquinoline-8-arsonic acid (4 g.) in fuming sulphuric acid (20 cc. of acid containing 10% of free SO_3), anhydrous potassium nitrate (8 g.) was added and the mixture heated on the boiling water-bath for 7 hours. The solution was then poured into water (100 cc.) and the reaction of the aqueous mixture adjusted with sodium hydroxide solution to pH 4: the pale yellow amorphous solid thus precipitated melted unsharply at 195° and on analysis was found to contain/

contain about 50% of unchanged chloroquinolylarsonic acid. After being heated on the water-bath with sulphuric acid and potassium nitrate (as used above) for another 7 hours, the product precipitated at the above isoelectric point had m.p. about 210° and still contained some unchanged initial-material. The product was heated with sulphuric acid and potassium nitrate under the above conditions for a further period of 7 hours: the compound then precipitated at pH 4 in stellate clusters of yellow needles melted sharply at 233-234° (decomp.) and was found to be pure nitro-chloroquinolylarsonic acid (Found: N, 8.1; Cl, 10.9; As, 22.7. $C_9H_6O_5N_2ClAs$ requires N, 8.4; Cl, 10.7; As, 22.55%). Yield, 2.2 g.

5-Chloro-6-nitroquinoline-8-arsonic acid is slightly soluble in hot water but practically insoluble in the usual neutral organic solvents. It dissolves readily in acetic acid. It is soluble in dilute aqueous sodium and ammonium hydroxides and in moderately concentrated mineral acids. A dilute acetic acid solution of the arsonic acid does not give a coloration with N/1000-iodine.

5-Piperidino-6-nitroquinoline-8-arsonic Acid (LXIV).

5-Chloro-6-nitroquinoline-8-arsonic acid (1 g.) was heated at 100° with piperidine (5 cc.) for 4 hours, and the mixture then added to water (10 cc.). The reaction of the orange solution was adjusted with hydrochloric acid to pH 5-6: the orange amorphous product thereby precipitated crystallised from dilute acetic acid in sheaves of long, orange-yellow, rectangular prismatic needles, m.p. 259-260° (decomp.); yield, 0.8 g. (Found: As, 19.9. $C_{14}H_{16}O_5N_3As$ requires As, 19.7%).

5-Piperidino-6-nitroquinoline-8-arsonic acid is slightly soluble in hot water and practically insoluble in the usual neutral organic solvents. It is readily soluble in acetic acid. It dissolves in dilute aqueous sodium and ammonium hydroxides and in moderately concentrated hydrochloric and sulphuric acids. A dilute acetic acid solution of the arsonic acid does not give a coloration with N/1000-iodine.

The yellow gelatinous sodium salt is readily precipitated when concentrated sodium hydroxide solution is added to a solution of the arsonic acid in dilute alkali: it dissolves on boiling but is reprecipitated in a gelatinous state on cooling. The following salts are precipitable from an aqueous solution/

solution of the ammonium salt: calcium salt, yellow, gelatinous, insoluble in hot water; barium salt, stellate clusters of yellow needles, soluble in hot water; magnesium and silver salts, yellow, gelatinous, insoluble in hot water; mercuric salt, sheaves of fine yellow needles, insoluble in hot water; lead salt, yellow, amorphous, insoluble in hot water; cupric salt, greenish-yellow, gelatinous, insoluble in hot water.

6-Nitro-5-hydroxyquinoline-8-arsonic Acid (LXV).

A solution of 5-chloro-6-nitroquinoline-8-arsonic acid (1 g.) in warm aqueous potassium hydroxide (10 cc. of d 1.3) was heated at 100° for 3 hours and then poured into water (10 cc.). The reaction of the dark orange-red solution was adjusted with hydrochloric acid to pH 4, and the orange-red crystalline arsonic acid thereby precipitated was redissolved in aqueous sodium carbonate solution, and filtered, and re-precipitated at the above isoelectric point with hydrochloric acid. The arsonic acid was thus obtained in stellate clusters of fine, slender, reddish-orange needles, m.p. 226-227° (vigorous decomp.); yield, 0.7 g. (Found: As, 24.0. $C_9H_7O_6N_2As$ requires As,/

As, 23.9%).

6-Nitro-5-hydroxyquinoline-8-arsonic acid is slightly soluble in hot water but practically insoluble in the usual neutral organic solvents. It is moderately easily soluble in acetic acid. It dissolves readily in dilute aqueous sodium and ammonium hydroxides and in moderately concentrated hydrochloric and sulphuric acids. A dilute acetic acid solution of the arsonic acid does not give a coloration with N/1000-iodine.

The sodium salt is slowly precipitated in stellate clusters of fine yellow needles when concentrated sodium hydroxide solution is added to a solution of the arsonic acid in dilute alkali. The following salts are precipitable in a gelatinous condition from an aqueous solution of the ammonium salt: barium and calcium salts, orange-yellow; silver and mercuric salts, orange (the latter is precipitated very slowly); lead salt, orange-brown: all are insoluble in hot water. The greenish-yellow gelatinous cupric salt is slightly soluble in hot water and crystallises in sheaves of fine slender needles on cooling. The magnesium salt is not precipitated in the cold but readily separates in an orange-yellow gelatinous state on boiling.

8-p-Tolylsulphonamido-5:7-diaminoquinoline (LXXIII)

8-p-Tolylsulphonamido-5:7-dinitroquinoline (10 g.) (prepared by nitration of 8-p-tolylsulphonamidoquinoline as described by Kaufmann and Zeller, Ber., 1917, 50, 1627) was dissolved in a mixture of alcohol (150 cc.) and ammonium hydroxide (30 cc.; d 0.880), and a rapid current of hydrogen sulphide passed through the cold solution. The mixture gradually became quite warm and after 1 hour it was boiled for a few minutes and cooled, and hydrogen sulphide again bubbled through for another hour. It was then poured into water (2 litres) and the yellow base thus liberated was freed from sulphur by dissolution in boiling hydrochloric acid (5%), filtration, and reprecipitation with aqueous sodium carbonate (yield, 8 g.). The base was further purified by crystallisation from alcohol and separated in sheaves of fine, long, flat, light brown needles, m.p. 207-208° (decomp.). (Found: C, 58.3; H, 5.0; N, 16.7; S, 9.6. $C_{16}H_{16}O_2N_4S$ requires C, 58.5; H, 4.9; N, 17.1; S, 9.8%).

8-p-Tolylsulphonamido-5:7-diaminoquinoline is insoluble in water. It is moderately easily soluble in alcohol and chloroform, sparingly soluble in benzene, and practically insoluble in light petroleum. It/

It is readily soluble in dilute sodium hydroxide solution, slightly soluble in ammonium hydroxide, but practically insoluble in aqueous sodium carbonate. The base dissolves readily in acetic, hydrochloric, nitric and sulphuric acids to give orange-red solutions. A dilute alcoholic or acetic acid solution of the base does not give a coloration with N/1000-iodine. The diazo-solution obtained from the hydrochloric acid solution on the addition of nitrous acid gives a crimson azo-dye on treatment with an alkaline solution of β-naphthol.

7:8-Triazolquinoline-5-arsonic Acid (LXXIV) and 7:8-Triazolquinoline (LXXV).

Finely powdered 8-p-tolylsulphonamido-5:7-diaminoquinoline (4.1 g.) was added to dilute hydrochloric acid (30 cc. of d 1.12 acid and 12 cc. of water) and the mixture cooled to 0°. A solution of sodium nitrite (2 g.) in water (5 cc.) was then gradually added so that the temperature did not rise about 5°. During the addition of sodium nitrite a very marked odour of p-tolylsulphonyl chloride was produced. The diazo-solution was allowed to stand for 1 hour and then/

then poured into a well-stirred solution of sodium arsenite (10.5 g.) in water (25 cc.) to which saturated copper sulphate solution (2.5 cc.) had been added. The mixture was kept at room-temperature for 5 hours and then warmed gently on the water-bath until evolution of nitrogen ceased. It was then made slightly alkaline with sodium hydroxide solution and boiled for a few minutes and filtered. The dark brown residue of insoluble by-products was extracted thrice with small quantities of aqueous sodium hydroxide (5%), and the reaction of the combined filtrates was adjusted with hydrochloric acid to pH 3-4; the light brown arsonic acid thereby precipitated was collected, redissolved in aqueous sodium carbonate solution, and reprecipitated with hydrochloric acid at pH 3-4. It was thus obtained in pale yellow microscopic needles, which were unmolten at 310° (Found: C, 37.1; H, 2.7; N, 19.3. $C_9H_7O_3N_4As$ requires C, 36.7; H, 2.4; N, 19.1%)*. Yield, 1.3 g.

7:8-Triazolquinoline-5-arsonic acid is insoluble in water and in the usual organic solvents. It dissolves readily in dilute sodium and ammonium hydroxide solutions and in moderately concentrated hydrochloric, sulphuric and nitric acids. An alcoholic or acetic acid suspension of the arsonic acid does not give a coloration with N/1000 iodine.

The/

* Owing to the great stability of this arsonic acid, it was not possible to carry out an arsenic estimation in the usual way.

The sodium salt is not precipitated when concentrated sodium hydroxide solution is added to a solution of the arsonic acid in dilute alkali. The following salts are precipitable from an aqueous solution of the ammonium salt: calcium salt, white, gelatinous; silver salt, buff, gelatinous; lead salt, white, gelatinous; cupric salt, green, gelatinous: all are insoluble in hot water. The mercuric salt is not precipitated in the cold but readily separates in a white gelatinous state on boiling. The barium and magnesium salts appear to be readily soluble and are not precipitated in the usual way.

When the acid mother liquors from which the above arsonic acid had been originally precipitated were neutralised with sodium hydroxide solution, a white gelatinous compound (0.6 g.) separated. It crystallised from hot water in stellate clusters of fine, slender, colourless needles, m.p. 256-257°. This compound proved to be 7:8-triazolquinoline (Found: C, 63.5; H, 3.4; N, 32.7. $C_9H_6N_4$ requires C, 63.5; H, 3.6; N, 32.9%).

7:8-Triazolquinoline is moderately easily soluble in hot water. It is readily soluble in acetic acid, slightly soluble in alcohol and chloroform, but practically insoluble in benzene and light petroleum. It dissolves in boiling dilute sodium carbonate and sodium hydroxide/

hydroxide solutions to about the same extent as it does in hot water; these solutions deposit fine slender needles on cooling. The base is soluble in hydrochloric, sulphuric and nitric acids. A dilute acetic acid solution of the base gives with N/1000-iodine a deep reddish-brown coloration which disappears on warming.

I

PREPARATION, HYDROLYSIS, AND REDUCTION OF THE FLUORO-, CHLORO-, AND BROMO-BENZYL BROMIDES.

BY

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AND

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XXXII.—*Preparation, Hydrolysis and Reduction of the Fluoro-, Chloro-, and Bromo-benzyl Bromides.*

By John Baldwin Shoesmith and Robert Henry Slater.

In previous communications (J., 1922, **121**, 1392; 1923, **123**, 2828; 1924, **125**, 1312, 2278) an account was given of the manner in which the reactivity of halogen atoms in various halogenated benzenoid derivatives is influenced by oxygen and by the hydrogen of a methyl group. The investigations have been continued with a view to discovering the effect of fluorine, chlorine, and bromine in such compounds. From the point of view of the principle of induced alternate polarities the halogens, except fluorine, appear to act as weakly negative "key-atoms" (as pointed out by Lapworth, *Mem. Manchester Phil. Soc.*, 1920, **64**, No. 3). The present investigation has shown that (1) fluorine is capable of inducing differences of reactivity very similar to, but smaller than those met with in the cases of the methoxybenzyl bromides (J., 1922, **121**, 1392) and the ω-bromoxylenes (J., 1924, **125**, 2278) and (2) chlorine and bromine induce still smaller differences, and the chloro- and bromo-benzyl bromides provide the first examples encountered in this series, in

which a change of reagent does not cause a change in the order of reactivity.

The order of ease with which the bromides lose their bromine as bromidion in solution in aqueous alcohol is: (1) *p*-fluorobenzyl bromide > benzyl bromide > *o*-fluoro- > *m*-fluoro-; (2) benzyl bromide > *p*-chloro- > *o*-chloro- > *m*-chloro-; (3) benzyl bromide > *p*-bromo- > *o*-bromo- > *m*-bromo-. The order of ease of reduction by hydriodic acid is: (1) *o*-fluoro- > *m*-fluoro- > benzyl bromide > *p*-fluoro-; (2) *o*-chloro- > *p*-chloro- > *m*-chloro- > benzyl bromide; and (3) *o*-bromo- > *p*-bromo- > *m*-bromo- > benzyl bromide. The differences of ease of hydrolysis are quite marked. Those of ease of reduction are small, but several repetitions of the experiments led to the same order of reactivity and, as is seen from the above, the order of ease of reduction of the chloro- and bromo-compounds is not analogous to that discovered in the cases of the methoxybenzyl bromides, the ω-bromoxylenes, and the fluorobenzyl bromides.

A series of experiments was undertaken to see if this non-reversal of the order of reactivity is due to any of the following causes: (*a*) removal of halogen from the nucleus, (*b*) liberation of iodine as follows: (1) $R{\cdot}C_6H_4{\cdot}CH_2Br + HI \longrightarrow R{\cdot}C_6H_4{\cdot}CH_2I + HBr$. (2) $2R{\cdot}C_6H_4{\cdot}CH_2I \longrightarrow R{\cdot}C_6H_4{\cdot}CH_2{\cdot}CH_2{\cdot}C_6H_4R$ (compare Silberrad, J., 1924, **125**, 2196), and (*c*) the more rapid formation of the iodide in the case of the para-isomerides and subsequent reduction of the iodides by hydriodic acid. The bromides were reduced for a long time, but the slight elimination of nuclear halogen then noted was not sufficient to reverse the discovered order of reactivity when correction was made for it. Reaction (2) is a photochemical one and does not take place in a dark thermostat such as was used in these experiments. In order to test (*c*), the bromobenzyl iodides were prepared; they were reduced by hydriodic acid at the same rate as the corresponding bromides. Thus it appears as if the conversion of the bromide into iodide is due to a mass-action effect and, owing to the concentration of hydriodic acid used, goes almost equally rapidly in all cases. Some of the iodides lose iodine more rapidly than their isomerides, and when a change in order of reactivity with change of reagent takes place alternating polar influences are very strong, *e.g.*, in the methoxybenzyl bromides.

An approximate estimate of the magnitude of the three influences which affect the reactivity of the bromine atom in the bromides, namely, the general (represented by *g*), the alternating (represented by *a*), and the so-called steric influence (represented by *s*), may be arrived at from considerations similar to those put forward by Flürscheim (J., 1909, **95**, 726). A comparison by means of reaction velocities, which is the most satisfactory method, is impossible,

because only during the hydrolysis of *o*- and *m*-fluorobenzyl bromides and also of the isomeric nitrobenzyl bromides (Shoesmith and Hetherington, J., 1924, **125**, 1316) were the reactions slow enough to give monomolecular velocity coefficients. The reduction of the bromides, moreover, is not the simple bimolecular reaction investigated by West in the case of bromomalonyl compounds (J., 1924, **125**, 710). The reciprocals of the times taken for half-completion of the reaction in the various cases may be used to obtain the necessary comparison.

If K_o, K_m, K_p and K_u represent the reciprocals of the times taken for half-completion of the reactions for the ortho-, meta-, para- and unsubstituted compounds, respectively, the manner in which the velocities of the reactions are affected by the three influences mentioned above may be expressed by the following equations : *

$$\log_{10} K_o = \log_{10} K_u + g + a + s.$$
$$\log_{10} K_m = \log_{10} K_u + g - a.$$
$$\log_{10} K_p = \log_{10} K_u + g + a,$$

and hence

$$g = \tfrac{1}{2}(\log_{10} K_m + \log_{10} K_p - 2 \log_{10} K_u),$$
$$a = \tfrac{1}{2}(\log_{10} K_p - \log_{10} K_m),$$

and $$s = \log_{10} K_o - \log_{10} K_p.$$

Obtained from graphs plotted from observations recorded in this and previous communications, the reciprocals of the various times taken for half-hydrolysis (x) and half-reduction (y) at the temperatures stated are summarised in Table I, on which the following remarks are based :

The general effect. The order in which the atoms or groups affect the reactivity of the bromine (*a*) towards hydrolysing agents in a general way is OMe, Me, F, Cl, Br, CO_2H, NO_2, ranging from the strongly enhancing methoxy-group to the strongly retarding nitro-group, and (*b*) towards hydrogen iodide is OMe and Me, Cl, Br, CO_2H, and F. In the latter case, only the methoxy- and the methyl-group have an appreciable general influence on the reduction velocity. The strong general influence of the methoxy-group is noteworthy.

The alternating effect. The order in which alternation towards hydrolysing agents is produced is similar to that given above (*a*); in this case, however, the magnitudes of the effect may be compared. The order is OMe > F > Me > Cl > Br > CO_2H > NO_2. The differences observed in the first five cases, *i.e.*, negative groups, have positive values, whilst in the last two, *i.e.*, positive groups, they have negative values. For reduction, the order is OMe and Me > CO_2H > F, Cl,

* Logarithms are used in order that the expressions shall finally involve a product or a quotient as the case may be.

TABLE I.

Compounds.	K_o.		K_m.		K_p.		g.		a.		s.	
	x.	y.	x.	y.	x.	y.	Hyd.	Red.	Hyd.	Red.	Hyd.	Red.
Methoxybenzyl bromides.	<33·3 (60°)	0·107 (25°)	0·47	12·5	>33·3*	0·00	>1·54	$+\infty$	0·92	$-\infty$	nil	∞
ω-Bromoxylenes.	2·17 (60°)	>0·00 (25°)†	0·77	0·15	2·78	0·00	0·68	$+\infty$	0·28	∞	−0·11	∞
Fluorobenzyl bromides.	0·28 (60°)	0·00 (25°)	0·20	0·00	0·91	0·00	−0·39	—	0·33	—	−0·51	—
Fluorobenzyl bromides.	1·0 (76°)	0·27 (101°)	0·71	0·22	4·0	0·18	−0·22	0·00	0·37	−0·05	−0·6	0·18
Chlorobenzyl bromides.	0·85 (76°)	0·36 (101°)	0·65	0·24	1·8	0·31	−0·60	0·27	0·22	+0·05	−0·32	0·07
Bromobenzyl bromides.	0·84 (76°)	0·36 (101°)	0·71	0·23	1·43	0·29	−0·67	0·22	0·15	+0·05	−0·23	0·09
Nitrobenzyl bromides.	0·29 (76°)	—	0·33	—	0·29	—	−1·69	—	0·028	—	nil	—
ω-Bromotoluic acids.	—	—	0·72 (76°)	0·27 (110°)	0·56	0·65	−1·07	+0·06	0·055	+0·19	—	—

Benzyl bromide x = 0·67 (60°), 2·17 (76°). y = 0 (25°), 0·20 (101°), 0·39 (110°).

* The extraordinary rapidity with which *p*-methoxybenzyl bromide is hydrolysed in aqueous alcoholic solution suggests that this figure should be much greater and hence g and a for hydrolysis approach those for reduction, *i.e.*, ∞.

† ω-Bromo-*o*-xylene is definitely but very slowly reduced at 25°. A definite value for $K_o(y)$ cannot be given, but s (red.) must be large on account of the stability of ω-iodo-*p*-xylene at 25° in presence of hydrogen iodide.

and Br. Here the differences in the cases of the OMe, Me and F compounds have positive values and in the other cases negative values. According to the principle of induced alternate polarities the sign of the difference is determined by the positive or negative character of the substituents, and the reduction of the chloro- and bromo-benzyl bromides is the only case so far met with in this series in which the sign of the difference is other than was expected.

The steric effect. The values obtained for *s* show that this effect in some cases diminishes and in other cases increases the reactivity, the former being observed in the hydrolyses, the latter in the reductions. Therefore the retarding of the reactions cannot be due to steric hindrance as normally conceived, and the results justify the conclusion that there is a disturbing factor governing the reactivity of ortho-compounds (see Lapworth and Shoesmith, J., 1922, **121**, 1394).

The influence of atoms or groups of atoms having the same electronic shell may be compared also, since the manner in which the methyl and the methoxy-group and the fluorine atom each affect the reactivity of the bromine atom of the $-C_6H_4\cdot CH_2Br$ group has been investigated. The order of potency is OMe > Me > F, except in the case of alternation of hydrolysis, for which the order is OMe > F > Me.

Experimental.

Preparation of the Isomeric Chlorobenzyl Bromides.—The most reliable method of preparing the ortho- and para-isomerides is indicated by the scheme :

$$C_6H_4Cl\cdot CH_3 \xrightarrow{Cl} C_6H_4Cl\cdot CHCl_2 \xrightarrow{HCO_2H} C_6H_4Cl\cdot CHO$$

$$\downarrow KOH$$

$$C_6H_4Cl\cdot CH_2Br \xleftarrow{HBr} C_6H_4Cl\cdot CH_2\cdot OH$$

The appropriate chlorotoluene, mixed with 5% of its weight of phosphorus pentachloride, was chlorinated at 160° until the required increase in weight had taken place. The oil thus obtained was boiled with twice its bulk of 98% formic acid (*d* 1·20) for ½ hour, the mixture poured into an excess of cold water, and the oil separated, dissolved in ether, washed twice with water, and once with aqueous sodium hydroxide. The aldehyde was precipitated as the bisulphite compound, which was carefully washed with ether, dissolved in water, and the pure aldehyde liberated by the addition of excess of sodium carbonate. It was converted into the alcohol (*o*-, m. p. 70°; *p*-, m. p. 72°) by treatment with 25% alcoholic potassium hydroxide, and this into the bromide by means of hydrogen bromide in benzene solution.

o-*Chlorobenzyl Bromide* is an oil, b. p. 102°/9 mm. (Found: Br, 39·05. C_7H_6ClBr requires Br, 38·9%). *p*-Chlorobenzyl bromide has m. p. 51° (compare Jackson and Field, *Ber.*, 1878, **11**, 905) (Found: Br, 38·7%).

m-*Chlorobenzyl Bromide.*—*m*-Nitrobenzaldehyde was converted into *m*-chlorobenzaldehyde (Erdmann and Schwechten, *Annalen*, 1890, **260**, 59), b. p. 213—214°, and this was reduced to the alcohol in alcoholic potassium hydroxide. The alcohol had b. p. 242°, not 234° as stated by Mettler (*Ber.*, 1905, **38**, 1749), and from it m-*chlorobenzyl bromide*, an oil of b. p. 109°/10 mm. (Found: Br, 38·8%), was obtained as before.

The Isomeric Bromobenzyl Bromides.—Each of these compounds was prepared by passing a stream of air through a weighed quantity of bromine into the appropriate, boiling bromotoluene. The product was distilled under diminished pressure, the portion passing over between 120° and 140°/12—16 mm. being collected, cooled, and when solid recrystallised from alcohol. The bromides thus obtained had m. p.'s: *o*-, 31°; *m*-, 40°; and *p*-, 63° [Found: hydrolysable Br, (*o*-) 32·1, (*m*-) 31·8, (*p*-) 31·8. Calc. for $C_7H_7Br_2$, hydrolysable Br, 32·0%] (compare Jackson, *Ber.*, 1876, **9**, 932).

The *isomeric bromobenzyl iodides* were prepared by boiling aqueous acetone solutions of the corresponding bromides with rather more than the calculated quantity of potassium iodide for ½ hour. The mixture was then poured into water, and the iodide recrystallised from light petroleum. o-*Bromobenzyl iodide* crystallises in shining, white needles, m. p. 47° (Found: I, 42·75. C_7H_6BrI requires I, 42·7%). m-*Bromobenzyl iodide* crystallises in white, six-sided prisms, m. p. 42° (Found: I, 42·8%). *p*-Bromobenzyl iodide crystallises in white needles, m. p. 73° (Hantzsch and Schultze, *Ber.*, 1896, **29**, 2253, give 80—81°) (Found: I, 42·9%).

[With R. H. SLATER.]

Preparation of the Isomeric Fluorotoluenes.—A solution of the requisite toluidine (25 g.) in a mixture of 30 c.c. of concentrated sulphuric acid and 80 c.c. of water was cooled to — 5° and diazotised with 20 g. of sodium nitrite dissolved in 50 c.c. of water, the temperature being kept below 5°. The solution of the diazotised base was added to about 400 c.c. of commercial hydrofluoric acid (50—60%) in a 1,500 c.c. brazed spun-copper flask surrounded by ice, after which the flask was fitted, by means of a cork, with a copper reflux condenser and very carefully warmed on a water-bath for about an hour (alternatively, the mixture may be left at room temperature for about 16 hours). The condenser was then reversed and the flask heated directly. The mixture of fluorotoluene, hydrofluoric acid,

and cresol that distilled was collected in a copper beaker containing 300 c.c. of 30% aqueous sodium hydroxide solution surrounded by a good freezing mixture. When all the fluorotoluene had distilled, the *alkaline* mixture in the beaker was extracted thrice with ether, the combined extracts were de-emulsified by saturated ammonium sulphate solution and dried over anhydrous sodium sulphate, and the ether was evaporated. In each case the fluorotoluene distilled at 113—118° and the yield was 15—16 g. (65%) (compare Holleman and Beekman, *Rec. trav. chim.*, 1904, **23**, 238).

Preparation of the Isomeric Fluorobenzyl Bromides.—Each fluorotoluene (80 g. in four lots) was brominated by volatilising bromine (30 g.) in a slow stream of dry air and passing the vapour into the boiling fluorotoluene (20 g.); by using small quantities nuclear substitution was avoided. The brominated oil was boiled with formic acid (*d* 1·20; *ca.* 2 vols.) for 6 hours, fluorobenzotribromide and fluorobenzylidene bromide being thus converted into fluorobenzoic acid and fluorobenzaldehyde, respectively, and some of the fluorobenzyl bromide into fluorobenzyl alcohol. The mixture was poured into a large excess of water, and the oil was separated, and washed in ethereal solution with 10% aqueous sodium hydroxide until free from fluorobenzoic and formic acids. After complete removal of the fluorobenzaldehyde with freshly prepared sodium bisulphite solution—the aldehydes and the benzyl bromides form constant-boiling mixtures—the ethereal solution was dried over anhydrous sodium sulphate, and the ether distilled. The residual oil was saturated in benzene solution with dry hydrogen bromide to convert any fluorobenzyl alcohol into the bromide, and after the removal of the benzene the fluorobenzyl bromide was fractionally distilled in a vacuum in the apparatus described by Widmer (*Helv. Chim. Acta*, 1924, **7**, 52). o-*Fluorobenzyl bromide* had b. p 84—85°/15 mm.; m-*fluorobenzyl bromide*, 77°/12 mm.; and p-*fluorobenzyl bromide*, 85°/15 mm. [Found: Br, 42·4 (*o*-); 42·0 (*m*-); 42·1 (*p*-). C_7H_6FBr requires Br, 42·3%].

Hydrolysis of the Bromides.—Twenty c.c. of a standard solution (105 c.c.) of the bromide in absolute alcohol were placed in a standard 25 c.c. flask, 5 c.c. of water added, and the volume was made exactly 25 c.c. by adding absolute alcohol. The whole was thoroughly mixed, immersed in the vapour of boiling carbon tetrachloride for a definite time, and then poured into a large volume of water. The liberated hydrobromic acid was titrated directly with standard aqueous sodium hydroxide and methyl-red. The results are in Table II, where w represents the total weight of benzyl bromide used, x the percentage changed, and t the time of hydrolysis in hours.

TABLE II.

	Ortho-compounds.			Meta-compounds.			Para-compounds.		
F:	w = 0·5218 g.			0·5456 g.			0·5102 g.		
Cl:	0·5600 ,,			0·5441 ,,			0·5418 ,,		
Br:	0·6702 ,,			0·6600 ,,			0·6693 ,,		
	x.			x.			x.		
t.	F.	Cl.	Br.	F.	Cl.	Br.	F.	Cl.	Br.
½	25	25	24	24	21	21	68	45	40
1	51	46	44	41	38	39	88	69	62
2	76	68	66	63	58	60	96	88	85
4	94	88	87	86	80	82	98	96	95
8	95	96	96	94	92	95	99	98	98

In Table III are results obtained in a similar manner in order to compare the hydrolysis of benzyl bromide, *m*-methoxybenzyl bromide,* the fluorobenzyl bromides, and the ω-bromoxylenes at 60·5° (b. p. of chloroform).

TABLE III.

	Fluorobenzyl bromides.			*m*-Methoxy-benzyl bromide.	Benzyl bromide.
	o.	*m*.	*p*.		
w.	0·5240	0·5252	0·5102	0·543	0·4610
t.	x.	x.	x.	x.	x.
½	9	7	26·5	14	22
1	20	11	48	29	39
2	33	23	69	49	59
4	56	41	90	72	80
8	76	63	95	88	88
16	91	81	96	—	—

Reduction of the Bromides.—Reduction at 25° under the conditions described by Lapworth and Shoesmith and by Shoesmith and Slater (*loc. cit.*) being so slow that satisfactory results could not be obtained, the bromides were reduced in a thermostat at 101°. Ten c.c. of a standard solution of the bromide in 50 c.c. of glacial acetic acid were placed in the 25 c.c. standard flask, 10 c.c. of freshly distilled hydriodic acid (*d* 1·680) added, the volume was made up to 25 c.c. with glacial acetic acid, and the whole thoroughly mixed, and kept in the thermostat for a definite time. The percentage reduction was then estimated as in previous cases.

The concentration of the hydriodic acid and the amount of iodine in it both influence the rate of reduction, and therefore a series of reductions was carried out on the same day with the same hydriodic acid. The results are in Table IV, t and x having the same significance as before.

* At all temperatures, in aqueous alcoholic solution, *p*-methoxybenzyl bromide is completely, and the *o*-compound almost completely, hydrolysed in 2½ minutes.

TABLE IV.

	Ortho-compounds. x.			Meta-compounds. x.			Para-compounds. x.		
t.	F.	Cl.	Br.	F.	Cl.	Br.	F.	Cl.	Br.
1½	29·5	32	30 (31)	23	23	23 (21)	22·5	28	26 (27)
3	45	53	53 (54)	37	43	43 (41)	34	49	47 (47)
6	65	76	74 (76)	63	64	61 (60)	57	68	64 (65)

Benzyl bromide : $x = 25$, 38, and 61 for $t = 1\frac{1}{2}$, 3, and 6, respectively.

The figures in brackets represent the reduction of the bromobenzyl iodides. In Table V are the results of reductions of the bromides made at a higher temperature (110°) in order to determine the amount of nuclear halogen eliminated.

TABLE V.

	Ortho-compounds. x.		Meta-compounds. x.		Para-compounds. x.	
t.	Cl.	Br.	Cl.	Br.	Cl.	Br.
2	76	77	57	57	70	72
4	91	91	81	79	88	88
8	102	100	97	97	98	97
18	104	103	100	100	101	101

p-Chlorobenzyl iodide (m. p. 65°) was isolated from the reaction of *p*-chlorobenzyl bromide and hydrogen iodide in glacial acetic acid at 25° (compare Shoesmith and Slater, *loc. cit.*), but, owing to the dilution at which the reductions had to be carried out, end-products in the other cases could not be separated. The figures obtained, however, show that the reaction in the cases of the meta- and para-isomerides was quite normal in that it first involved conversion of the bromide into iodide and subsequent reduction of this to the halogenated toluene.

The iodobenzyl bromides have not been examined, since in a former communication we showed that hydrogen iodide reduces iodotoluene to toluene.

The authors gratefully acknowledge the many valuable suggestions made by Dr. W. O. Kermack, especially with regard to the formulæ on pp. 216, and the receipt of a grant from the Earl of Moray Research Fund. They also thank the Trustees of the Carnegie Trust for the Universities of Scotland for a scholarship which enabled one of them (R. H. S.) to take part in this research.

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The Isomeric Fluorobenzaldehydes and their Derivatives. By JOHN BALDWIN SHOESMITH, CHARLES EDWIN SOSSON, and ROBERT HENRY SLATER.

ORTHO- and para-fluorobenzaldehydes have now been separated from the bisulphite compounds obtained as by-products in the preparation of the corresponding fluorobenzyl bromides (Shoesmith and Slater,

this vol., p. 220). The b. p.s are : *o*-, 175°/760 mm.; *p*-, 181·5°/763 mm. (compare Rinkes, *Chem. Weekblad*, 1919, **16**, 206). *m*-Fluorobenzaldehyde could not be obtained pure in this way, but was prepared by passing a current of pure dry hydrogen for 24 hours into a commercial xylene solution of *m*-fluorobenzoyl chloride (Meyer and Hub, *Monatsh.*, 1910, **31**, 9344) of b. p. 73·4°/11 mm. in contact with palladinised barium sulphate (compare Rosenmund, *Ber.*, 1918, **51**, 591). An apparently theoretical yield of the aldehyde bisulphite compound, when dried over phosphoric oxide, became a 60% yield, the same phenomenon being noted with benzoyl chloride. m-*Fluorobenzaldehyde* is a colourless oil of b. p. 173°/760 mm. (Found : F, 14·8. C_7H_5OF requires F, 15·3%). The fluorine content was estimated by mixing 0·20 g. of the aldehyde with 0·40 g. of starch and placing small quantities of this powder and of sodium peroxide (10 g. in all were used) alternately in a Parrs bomb. The bomb was closed, well shaken, and fired in the usual way. This modification of Hahn and Reid's method (*J. Amer. Chem. Soc.*, 1924, **46**, 1652) was necessary because the aldehyde, starch, and sodium peroxide explode spontaneously when mixed in an open vessel. The fluorine was then estimated as calcium fluoride in the usual manner.

The fluorobenzaldoximes crystallise from ligroin in fine, white plates : *o*-, m. p. 63° (Rinkes gives 62·6°) (Found : N, 10·2%); *m*-, m. p. 63° (Found : N, 10·1%); *p*-, m. p. 86·5° (Rinkes gives 81·2°) (Found : N, 10·2%).

The *fluorobenzaldehydephenylhydrazones* crystallise from aqueous alcohol in white plates : *o*-, m. p. 89·5° (Found : N, 13·2. $C_{13}H_{11}N_2F$ requires N, 13·1%); *m*-, m. p. 114° (Found : N, 13·15%); *p*-, m. p. 147° (Found : N, 13·2%).

The *fluorobenzaldehyde*-p-*nitrophenylhydrazones* crystallise from dilute acetic acid in fine, orange, rectangular needles : *o*-, m. p. 205° (Found : N, 16·3. $C_{13}H_{10}O_2N_3F$ requires N, 16·3%); *m*-, m. p. 202° (Found : N, 16·2%); *p*-, m. p. 212° (Found : N, 16·4%).

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III

POLARITY EFFECTS IN THE ISOMERIC ω-BROMOXYLENES AND ISOMERIC IODOTOLUENES.

BY

JOHN BALDWIN SHOESMITH

AND

ROBERT HENRY SLATER.

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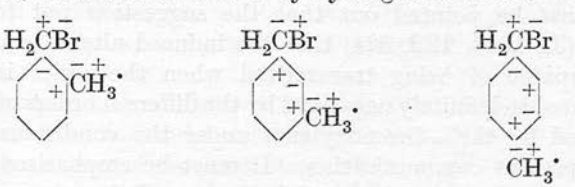
Reprinted from the Journal of the Chemical Society,
Volume 125, 1924.

CCCV.—*Polarity Effects in the Isomeric ω-Bromoxylenes and Isomeric Iodotoluenes.*

By John Baldwin Shoesmith and Robert Henry Slater.

The investigations of this series (Lapworth and Shoesmith, J., 1922, **121**, 1392; Shoesmith, J., 1923, **123**, 2838; Shoesmith, Hetherington, and Slater, this vol., p. 1312), which have so far been confined to the influence which oxygen exerts as a "key-atom" on halogen atoms in various benzenoid compounds, have been extended and the influence of the hydrogen atoms in a methyl group in such compounds ascertained.

By the same methods as were employed in the first investigations, it has now been possible to show that the isomeric ω-bromoxylenes react in an anticipated order. The order of ease of hydrolysis should be *p* and *o*>*m* and of reduction by hydrogen iodide *m*>*o* and *p*.



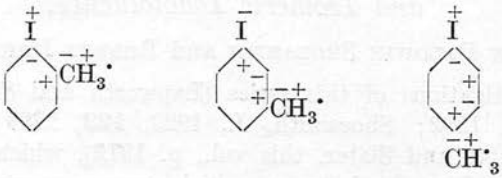
* 10-*Chloro*-5-*benzoyl*-5 : 10-*dihydrophenarsazine*, $C_6H_5{\cdot}CO{\cdot}N(C_6H_4)_2AsCl$, has not been previously described. It is prepared from 10-chloro-5 : 10-dihydrophenarsazine in an excess of boiling benzoyl chloride, diluted with dry xylene, for 10 hours. After removal of excess xylene the dark coloured viscous oil is allowed to stand for 2 days and the solid material filtered off. The substance is obtained in colourless, hard crystals, m. p. 180—181° on crystallisation from benzene (Found : As = 19·7. $C_{19}H_{13}ONClAs$ requires As = 19·64%).

Experiment has fully borne out this prediction. It has, in fact, been found that under conditions by which ω-bromo-*m*-xylene is almost quantitatively reduced by hydrogen iodide to *m*-xylene, the isomeric ω-bromo-*p*-xylene is converted into ω-iodo-*p*-xylene.

The complete order of hydrolysis is $p>o>m$, and of reduction $m>o>p$.

The general polar influence exerted by the methyl group is quite marked and all the ω-bromoxylenes are more easily hydrolysed than the unsubstituted benzyl bromide. It must also be pointed out that the meta- and ortho-isomerides are more reactive than the unsubstituted compound to both reagents. As expected, ω-bromo-*p*-xylene is more easily hydrolysed than ω-iodo-*p*-xylene.

The order of ease of reduction of the iodotoluenes, in which the halogens are one place nearer to the "key-atom" than those present in the ω-bromoxylenes, was again as expected, namely, *o* and $p>m$, the reverse of the previous order, except that the ortho-isomeride is the isomeride most easily reduced to toluene.



The meta-isomeride is reduced very slowly under the experimental conditions, and therefore this series differs slightly from that of the halogenated phenols, in which the meta-isomeride is not reduced.

The possibility of the formation of reactive molecules of quinonoid type in any of the compounds here investigated is remote and the two series of experiments are of importance by reason of their simplicity.

It must be pointed out that the suggestion put forward by Lowry (J., 1923, **123**, 824) that the induced alternate influence is only capable of being transmitted when the series is perfectly conjugated is definitely negatived by the different orders of reactivity displayed by the ω-bromoxylenes under the conditions described in the present communication. It must be emphasised, however, that the benzene ring is certainly a very efficient transmitter and possibly even an amplifier of induced polarity effects. In all probability, however, similar effects will not be observed in aliphatic compounds which contain a series of consecutive single bonds. This has recently been stressed by Lapworth (*Far. Soc. Disc.*, 1923, p. 505).

In addition, it is obvious that the tautomeric hydrogen hypothesis

of Thorpe (J., 1911, **99**, 2185, and onwards) cannot be extended to embrace the observations on the differences of ease of reducibility of the methoxybenzyl bromides (Lapworth and Shoesmith, *loc. cit.*) or of the halogen compounds described in the present communication.

EXPERIMENTAL.

Preparation of the ω-Bromoxylenes.—These were all obtained by a general method summarised as follows :—

$$\underset{\text{(I.)}}{CH_3\cdot C_6H_4\cdot NH_2} \longrightarrow \underset{\text{(II.)}}{CH_3\cdot C_6H_4\cdot CN} \xrightarrow[\text{alcohol}]{\text{Na in}} \underset{\text{(III.)}}{CH_3\cdot C_6H_4\cdot CH_2\cdot NH_2} \xrightarrow{HNO_2} \underset{\text{(IV.)}}{CH_3\cdot C_6H_4\cdot CH_2\cdot OH} \xrightarrow[\text{dry benzene}]{\text{HBr in}} \underset{\text{(V.)}}{CH_3\cdot C_6H_4\cdot CH_2Br}$$

The toluidines (I) were converted into the corresponding toluonitriles (II) in the usual manner, and the latter purified by distillation in steam. They had b. p., *o*- 202—204°, *m*- 210—212°, and *p*- 215—217°, respectively.

The nitriles were reduced to the tolylmethylamines (III) by means of sodium and alcohol (compare Kröber, *Ber.*, 1890, **23**, 1026; Sommer, *ibid.*, 1900, **33**, 1073). Sodium (100 gms.) was added, through an upright condenser, to a boiling solution of the nitrile (30 gms.) in perfectly dry alcohol (1 litre). When all the sodium had dissolved, the reduction mixture was diluted with water, acidified with hydrochloric acid, and the alcohol distilled in steam. The tolylmethylamine liberated from the residual acid solution by sodium hydroxide was distilled in steam, extracted from the distillate with ether, dried over sodium sulphate, and purified by distillation.

The yields of the *o*-, *m*-, and *p*-tolylmethylamines obtained by this general method were, *o*- 50%, *m*- 30%, and *p*- 60% of the expected quantity. They distilled at 200—202°, 198—200°, and 194—196°, respectively.

The corresponding tolylcarbinols (IV) were obtained from the amines by the addition of twice the necessary quantity of sodium nitrite to a solution of the base in an excess of dilute hydrochloric acid. Nitrogen was evolved at once. After 12 hours, the reaction was completed on the water-bath. The carbinol, removed from the reaction mixture with ether, was distilled in steam, and obtained pure from the ethereal extract of the distillate. The yields were *o*- 50%, *m*- 70%, and *p*- 40%. *o*-Tolylcarbinol, m. p. 33°, b. p. 112—114°/9 mm.; *m*-tolylcarbinol, b. p. 108—111°/10 mm.; *p*-tolylcarbinol, m. p. 60°. The poor yields of the ortho- and para-

isomerides are due to the fact that appreciable resinification took place during steam distillation of the tolylcarbinols.

The isomeric ω-bromoxylenes (V) were obtained from the corresponding tolylcarbinols by the usual method of saturating the dry benzene solution of the latter with dry hydrogen bromide. The *o*-isomeride, b. p. 102°/11 mm., m. p. 20°; *m*-, b. p. 97—99°/8 mm.; *p*-, b. p. 100°/9 mm., m. p. 35·5°.

Hydrolysis of the ω-Bromoxylenes at 60°.

The isomerides were hydrolysed at the temperature of the vapour of boiling chloroform, since this was found to be the most satisfactory temperature at which a series of results could be obtained. The method was that used in a previous communication (Shoesmith, Hetherington, and Slater, *loc. cit.*). The results are summarised in Table I,* where *t*, *w*, and *x* represent time in hours from commencement of experiment, weight of compound used, and percentage changed, respectively.

TABLE I.

	Ortho-compound.		Meta-compound.		Para-compound.		Benzyl bromide.		ω-Iodo-*p*-xylene.	
t.	*w*.	*x*.	*w*.	*x*.	*w*.	*x*.	*w*.	*x*.	*w*.	*x*.
½	0·1060	55	0·0980	25	0·0983	66	0·1093	22	0·1335	38·8
1	0·1064	77	0·0996	42	0·1074	87	0·1056	37	0·1146	63·1
2	0·1030	89	0·1037	64	0·1019	96	0·1100	59	0·1280	78·8
3	0·1022	94	0·1011	77	0·0997	100	0·1078	71	0·1291	84·7

Quantitative Reduction of the ω-Bromoxylenes by Hydrogen Iodide at 25°.

A solution of hydrogen iodide in glacial acetic acid was used which contained 0·70 gm. of HI per c.c. Approximately 0·5 gm. of the bromoxylene was dissolved in sufficient glacial acetic acid to make the volume 1 c.c. in a 5-c.c. ground glass-stoppered measuring cylinder. The reducing agent (4 c.c.) was then added and the cylinder immersed in the thermostat. The rate at which the reduction took place was determined as in a previous communication (Lapworth and Shoesmith, *loc. cit.*). The results are summarised in Table II, where *t*, *w*, and *x* have the same significance as before.

Identification of the Reduction Products.—Approximately 6 grams of the ω-bromoxylene and 45 c.c. of the reducing agent were used. The mixture was maintained at 25° for 40 hours and then poured into water. The solids which separated in the experiments with the ortho- and para-isomerides and also with benzyl bromide were filtered off, dried, and recrystallised from light petroleum. They

* For convenience, the results for ω-iodo-*p*-xylene (for preparation, see p. 2282) are included.

TABLE II.

	Ortho-compound. $w=0{\cdot}5862$ gm.	Meta-compound. $w=0{\cdot}5537$ gm.	
t.	x.	x.	
$\frac{1}{2}$	—	17·9	The para-isomeride did not reduce under these conditions, and iodine corresponding to 2 per cent. reduction was liberated from benzyl bromide.
$1\frac{1}{2}$	2·5	25·6	
$3\frac{1}{2}$	4·2	36·5	
7	8·2	50·8	
20	12·1	83·9	

proved to be the corresponding iodo-derivatives; ω-iodo-*o*-xylene, m. p. 33—34°, ω-iodo-*p*-xylene, m. p. 46—47° (compare Pavlovsky, *J. Russ. Phys. Chem. Soc.*, 1911, **43**, 214), and benzyl iodide, m. p. 24°. Estimations of the hydrolysable iodine confirmed this.

In addition to the solid which was obtained from the reduction product of the ortho-compound a small drop of oil was observed, but it was not possible to identify it owing to the very small quantity which separated out.

m-Xylene was isolated from the reduced meta-isomeride in the following way. The reduction mixture was poured into excess of water, decolorised by the addition of sodium thiosulphate, and the acid neutralised with sodium hydroxide. The whole was extracted with ether and from the ethereal extract an oil (1·5 gm.) was obtained which distilled between 135—150°. When redistilled, it boiled at 135—143°. It was identified by its density (0·857 at 16°) and its trinitro-derivative, m. p. 181—182°, which did not depress the melting point of an authentic specimen of trinitro-*m*-xylene.

Reduction of the Iodotoluenes at 25°.

The iodotoluenes were prepared from the toluidines. The ortho-isomeride distilled at 205° and the para at 211° (m. p. 35°).

In order to obtain the meta-isomeride perfectly free from ortho-compound, *m*-nitrotoluene was purified by means of ethyl oxalate and sodium methoxide as described by Reissert (*Ber.*, 1897, **30**, 1047). The pure *m*-nitrotoluene (m. p. 16°, b. p. 231°) was reduced with iron filings and a small quantity of acetic acid (see also Shoesmith, Hetherington, and Slater, *loc. cit.*). The toluidine, which distilled at 203°, was converted into *m*-iodotoluene, b. p. 213°, in the usual way. The explosions which took place during the last preparation, especially when it was carried out on a moderately large scale, are noteworthy, and small-scale experiments (10 gms. of *m*-toluidine) are to be recommended.

The iodotoluenes were reduced under the same conditions as were the ω-bromoxylenes, and in Table III, t is the time in days

from the commencement of the experiment, whilst w and x have the same significance as before.

TABLE III.

	Ortho-compound.	Meta-compound.	Para-compound.		Ortho-compound.	Meta-compound.	Para-compound.
	$w = 1{\cdot}2150$ gm.	$1{\cdot}1934$ gm.	$1{\cdot}2051$ gm.				
t.	x.	x.	x.	t.	x.	x.	x.
1	48·4	4·1	32·1	6	88·1	13·3	79·2
2	67·9	7·9	50·5	8	92·0	17·4	85·9
4	82·8	10·9	69·9				

From the *o*- and *p*- isomerides toluene was isolated by the method employed for the separation of *m*-xylene. It distilled at 105—118°, had density 0·876 at 16°, was quite free from halogen, and its trinitro-derivative (m. p. 81—82°) did not depress the melting point of an authentic specimen of trinitrotoluene.

Under the above conditions iodobenzene liberated iodine in a quantity which represented 10% reduction in 8 days.

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Volume 127, 1925.

The Reduction of Anethole Nitrosochloride by Stannous Chloride and Hydrochloric Acid. By JOHN BALDWIN SHOESMITH and ROBERT HENRY SLATER.

TWENTY grams of anethole nitrosochloride (see Orndorff and Morton, *Amer. Chem. J.*, 1900, **23**, 181) in 150 c.c. of chloroform (the only solvent in which the nitrosochloride is appreciably soluble) were vigorously stirred with 150 g. of stannous chloride dissolved in 200 c.c. of concentrated hydrochloric acid. The emulsion became transparent and yellow whilst its temperature gradually rose to 50°. After ½ hour's stirring, a yellow solid was precipitated and after 6 hours, precipitation was complete, the temperature of the mixture being that of the room. After filtration, the solid was dried over calcium chloride and potassium hydroxide, and unchanged nitrosochloride extracted with chloroform. The residue (m. p. 259° with decomp.) was the double compound of *anisylideneazine hydrochloride* and *stannic chloride*, $(MeO\cdot C_6H_4\cdot CH{:}N\cdot N{:}CH\cdot C_6H_4\cdot OMe,HCl)_2,SnCl_4$ (Found: Cl, 24·7; Sn, 13·1. $C_{32}H_{34}O_4N_4Cl_6Sn$ requires Cl, 24·45; Sn, 13·6%), which with water was decomposed into stannic oxide and anisylideneazine, m. p. 168° (to a liquid crystal which was converted into the isotropic liquid at 180°) (Found: N, 10·8. Calc., N, 10·45%). Anisylideneazine acts as a mono-acid base, and in solution in chloroform is converted by hydrogen chloride into *anisylideneazine hydrochloride*, $MeO\cdot C_6H_4\cdot CH{:}N\cdot N{:}CH\cdot C_6H_4\cdot OMe,HCl$, fine, yellow needles, m. p. 172°, which decompose at 177° (Found: Cl, 11·7. $C_{16}H_{17}O_2N_2Cl$ requires Cl, 11·6%) and give anisaldehyde with nitrous acid and the above double compound with stannic chloride.

The authors wish to acknowledge a grant from the Earl of Moray Research Fund which rendered the investigation possible.—EDINBURGH UNIVERSITY. [*Received, March 4th*, 1925.]

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V

POLARITY EFFECTS IN AROMATIC HALOGEN COMPOUNDS.

BY

JOHN BALDWIN SHOESMITH,

ARTHUR CLEMENT HETHERINGTON,

AND

ROBERT HENRY SLATER.

From the Transactions of the Chemical Society, 1924. Vol. 125.

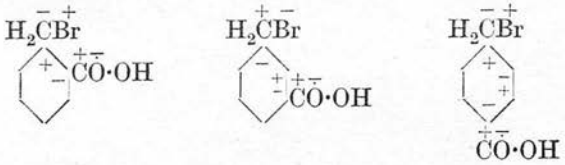
CLXIII.—*Polarity Effects in Aromatic Halogen Compounds.*

By John Baldwin Shoesmith, Arthur Clement Hetherington, and Robert Henry Slater.

The investigations of Lapworth and Shoesmith (J., 1922, **121**, 1392) and Shoesmith (J., 1923, **123**, 2828) have been continued and it has been found possible to predict differences in reactivity as regards hydrolysis and reduction by hydrogen iodide of the position isomerides of a number of halogen-substituted benzenoid compounds by a simple application of the principle of induced alternate polarities.

In addition to the induced alternate polarity influences in a molecule two other factors must be considered : (*a*) general polar influences, which are due to substituent atoms or groups and affect the molecule as a whole (Flürscheim, J., 1909, **95**, 718; Lapworth, *Mem. Manchester Phil. Soc.*, 1920, **64**, No. 3; Kermack and Robinson, J., 1922, **121**, 428; Lapworth and Shoesmith, *loc. cit.*; Robinson, *Ann. Reports*, 1922, 99), and (*b*) spatial and steric factors which operate generally in the aromatic series in the ortho-isomerides. When discussing differences in reactivity, it is therefore necessary to consider closely related compounds such as isomerides in order to eliminate (*a*) and meta- and para-isomerides to eliminate (*b*).

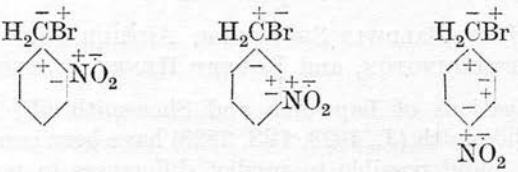
In the three isomeric ω-bromotoluic acids,



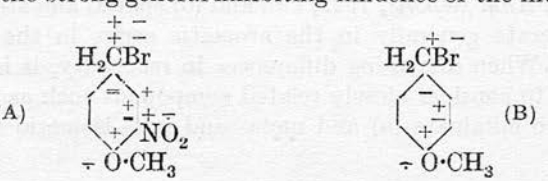
the bromine which has acquired an induced negative character is present in the meta-isomerides; thus the order of ease of hydrolysis should be $m>p$, and of reduction by hydrogen iodide, $p>m$, the reverse of that found in the methoxybenzyl bromides. Both these predictions were borne out by experiment (see Tables I and II).

Reduction of the carboxyl group under the conditions of the experiment is so small that it may be ignored.

Unsubstituted benzyl bromide is more easily hydrolysed (Table I) than any of the ω-bromotoluic acids. The introduction of the carboxyl group therefore stabilises the benzyl bromide molecule as a whole, a condition even more pronounced in the case of the nitrobenzyl bromides, where the general polar influence (*a*) is very marked. The ease of hydrolysis of the latter compounds still follows the same rule, and the meta is the most readily hydrolysed isomeride (Table III).

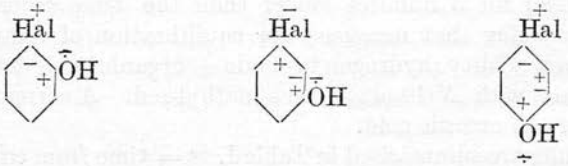


The introduction of the nitro-group into a very reactive molecule such as *p*-methoxybenzyl bromide diminishes the reactivity of the latter to the order of that of the unsubstituted compound. It is thus seen that the restriction that differences in reactivity can be predicted only for very closely related compounds such as isomerides is necessary, otherwise 3-nitro-4-methoxybenzyl bromide (A) (which was the compound investigated) might be expected to be more reactive than the very reactive *p*-methoxybenzyl bromide (B) in virtue of the enhanced induced negative character of the bromine atom in the former. This induced effect is more than balanced by that of the strong general inhibiting influence of the nitro-group.



It has also been possible to show that 3-nitro-4-hydroxybenzyl bromide is more easily hydrolysed by aqueous alcohol than 3-nitro-4-methoxybenzyl bromide (Table III). Hydriodic acid reduces the nitro-group and therefore the change of order of reactivity with change of reagent could not be investigated.

The halogen atoms in the halogenated phenols are to be regarded as being situated one place nearer the " key-atom " than are those in the corresponding methoxybenzyl bromides. Thus the order of ease of reduction of such phenols should be, and actually is, p and $o > m$, the reverse of that already found in the latter series.



The order in which halogen atoms are removed from such compounds is p-iodo > o-iodo > p-bromo > o-bromo > p-chloro (Tables IV and V). The meta-isomerides show no tendency to reduce.* 4-Iodoresorcinol reduces far more rapidly than the iodophenols. It is, of course, recognised that in this series a quinonoid change could assist or addition of reducing agent might precede reduction, but the ultimate result is that which would be expected on the theory of induced alternate polarities.

EXPERIMENTAL.

[With A. C. HETHERINGTON.]

Bromotoluic Acids.—The ω-bromotoluoyl bromides from which the corresponding ω-bromotoluic acids were obtained were prepared by Davies and Perkin's method (J., 1922, **121**, 2202). The reactions are summarised as follows.

$$C_6H_4Me{\cdot}CO_2H \xrightarrow{SOCl_2} C_6H_4Me{\cdot}COCl \xrightarrow{Br} CH_2Br{\cdot}C_6H_4{\cdot}COBr \xrightarrow{HCO_2H} CH_2Br{\cdot}C_6H_4{\cdot}CO_2H.$$

ω-Bromo-*m*-toluoyl bromide has b. p. 160—165°/14 mm. and m. p. 23—25°. *ω-Bromo-p-toluoyl bromide* has b. p. 165—170°/12 mm. and m. p. 39—40° (Found : Br = 54·55. $C_8H_6OBr_2$ requires Br = 57·55 per cent. The low result is due to hydrolysis by atmospheric moisture).

* Franzen and Stäuble (*J. pr. Chem.*, 1921, [ii], **103**, 352) have prepared 3-chloro-α-naphthol from 2 : 3 : 4-trichloro-α-naphthol by reduction with hydriodic acid. The resulting monochloro-compound is quite stable to the reducing agent.

A mixture of 25 grams of the ω-bromotoluoyl bromide and 300 c.c. of 80 per cent. formic acid (*d* 1·2) was warmed at 30—35° for 1 hour and poured into water, and the precipitated acid was dried and crystallised from benzene. The m. p.'s of the acids are, *o*- 146°, *m*- 150°, and *p*- 223° (compare Zalkind, *J. Russ. Phys. Chem. Soc.*, 1914, **46**, 508; Zalkind and Semenov, *ibid.*, p. 512).

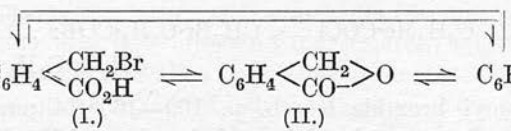
Hydrolysis of the ω-Bromotoluic Acids.—Approximately 0·10 gram of the acid was dissolved in 20 c.c. of absolute alcohol, 5 c.c. of water were added, and the volume was made up to 25 c.c. with alcohol. The solution was heated at 76° (vapour of boiling carbon tetrachloride) for 5 minutes longer than the time recorded, the extra time being that necessary for equilibration of temperature, and the total acidity (hydrogen bromide + organic acid) determined by titration with *N*/10-alkali and methyl-red. A correction was applied for the organic acid.

The results are summarised in Table I. t = time from commencement of experiment in hours. w = weight of compound used in grams. x = percentage changed.

TABLE I.

	o-Compound.		*m*-Compound.		*p*-Compound.		Benzyl bromide.	
t.	*w*.	*x*.	*w*.	*x*.	*w*.	*x*.	*w*.	*x*.
½	0·1068	5·0	0·1019	27	0·1076	13	0·0804	57
1	0·1158	7·0	0·1010	42	0·1049	30	,,	81
2	0·1031	4·9	0·1207	70	0·1126	54	,,	92
4	0·1089	4·7	0·1001	87	0·1110	74	,,	96
8	0·1126	4·8	0·1149	90	0·1032	83	,,	97
16	—	—	0·1154	94	0·1207	90	0·0975	99
32	—	—	0·1199	98	0·0998	94	—	—

In the case of ω-bromo-*o*-toluic acid, x denotes, not the percentage of acid hydrolysed, but that calculated from the excess titration over the quantity required for neutralisation of the carboxyl group. The fall from the maximum is due to the following changes:



(I.) (II.) (III.)

The hydrolysis of ω-bromo-*o*-toluic acid (I) to ω-hydroxy-*o*-toluic acid (III) is accompanied by the formation of hydrobromic acid and phthalide (II). In the titrations, which estimate both the halogen acid and the carboxylic acid, the values fall until equilibrium is reached in the production of phthalide. This is due to the spatial influences classified under heading (*b*).

Reduction of the Isomeric ω-Bromotoluic Acids at 110° *by Hydriodic*

Acid.—Approximately 0·10 gram of the acid, dissolved in 15 c.c. of glacial acetic acid, was heated with 10 c.c. of constant-boiling hydriodic acid in the vapour of boiling toluene. After a definite interval the liberated iodine was titrated with thiosulphate. A blank experiment to ascertain the amount of air oxidation was carried out on each occasion.

The results are summarised in Table II; t, w, and x have the same significance as before.

TABLE II.

	o-Compound.		*m*-Compound.		*p*-Compound.		Benzyl bromide.	
t.	*w.*	*x.*	*w.*	*x.*	*w.*	*x.*	*w.*	*x.*
1½	0·1026	11	0·1010	24	0·1044	49	0·1518	34
3	0·1223	24	0·1003	43	0·1033	73	0·1549	55
6	0·1161	42	0·1001	68	0·1001	91	0·0900	78
12	0·0931	51	0·1066	86	0·0901	99	0·1103	92

The behaviour of the *o*-compound is again abnormal owing to phthalide formation.

Reduction of the isomeric toluic acids for 6 hours under the same conditions as those in the previous experiment takes place to the following extents: *o*- 0·16, *m*- 0·49, *p*- 0·43 per cent.

Corrections for such small quantities have not been applied to the bromo-acid reduction figures.

o-*Nitrobenzyl Bromide.*—*o*-Nitrobenzyl chloride (Haeussermann and Beck, *Ber.*, 1892, **25**, 2445) (1 part) was boiled with concentrated aqueous sodium acetate (2 parts) and the *o*-nitrobenzyl acetate converted into *o*-nitrobenzyl alcohol by boiling 50 per cent. sulphuric acid. This alcohol was crystallised from hot water and, when mixed with the calculated quantity of phosphorus pentabromide, readily gave o-*nitrobenzyl bromide*, light yellow plates, m. p. 45·5°, from light petroleum (Found: Br = 36·82. $C_7H_6O_2NBr$ requires Br = 37·00 per cent.).

m-Nitrobenzyl bromide was prepared from *m*-nitrobenzyl alcohol and hydrogen bromide in dry benzene, two layers forming. The crystals that separated from the lower layer and the further quantity obtained by evaporating the benzene were recrystallised from light petroleum; the product had m. p. 57°.

p-Nitrobenzyl bromide, prepared by Lyons and Reid's method (*J. Amer. Chem. Soc.*, 1917, **39**, 1729) and recrystallised several times from light petroleum to remove benzal bromide, melted at 98·5°.

3-*Nitro*-4-*hydroxybenzyl bromide* was obtained from the corresponding alcohol, which was prepared by Stoermer and Behn's method (*Ber.*, 1901, **34**, 2459). The alcohol crystallised from water in bright yellow needles, m. p. 97°. A solution of these in the

minimum of dry benzene was saturated with dry hydrogen bromide, the benzene evaporated, and the residue crystallised from light petroleum, 3-*nitro*-4-*hydroxybenzyl bromide* being obtained in yellow prismatic needles, m. p. 82° (Found: Br = 34·46. $C_7H_6O_3NBr$ requires Br = 34·44 per cent.).

3-*Nitro*-4-*methoxybenzyl bromide*, prepared by methylating 3-nitro-4-hydroxybenzyl alcohol with methyl iodide and potassium hydroxide, recrystallising the product from hot water, and converting it (m. p. 69°) into the bromide as above, crystallised from light petroleum in pale yellow needles, m. p. 108° (Found: Br = 32·84. $C_8H_8O_3NBr$ requires Br = 32·49 per cent.).

These bromides were hydrolysed under the same conditions as the ω-bromotoluic acids. The hydrobromic acid liberated was titrated with *N*/10-caustic alkali as before, except in the case of 3-nitro-4-hydroxybenzyl bromide, where *N*/20-ammonium hydroxide was used and any hydrolysis of the unchanged bromide during the titration avoided.

The results are summarised in Table III.

TABLE III.

Nitrobenzyl bromides

	Ortho.		Meta.		Para.		3-Nitro-4-methoxybenzyl bromide.	
t.	*w*.	*x*.	*w*.	*x*.	*w*.	*x*.	*w*.	*x*.
½	0·1267	10·8	0·1226	12·0	0·1168	11·0	0·1067	73·2
1	0·1190	19·2	0·1037	23·0	0·1045	19·5	0·1008	90·2
2	0·1022	32·4	0·1083	38·4	0·1147	32·8	0·1023	95·5
4	0·1010	54·7	0·1171	59·3	0·1015	54·6	0·1116	97·1
8	0·1081	78·9	0·1196	85·1	0·1146	78·9	0·1037	98·7
16	0·1040	93·9	0·1194	96·2	0·1068	95·3	0·1038	99·3
32	0·0998	99·1	0·1176	99·8	0·1041	99·5	0·1039	99·8

3-Nitro-4-hydroxybenzyl bromide.

t	¼	½	1	2
w	0·1008	0·1024	0·1031	0·1028
x	85	96	99	100

[With ROBERT HENRY SLATER.]

The *o*- and *p*-halogenated phenols were prepared from the corresponding aminophenols by the usual methods.

m-Chlorophenol was most conveniently obtained from *m*-chloronitrobenzene, 100 grams of which at 50° were dropped into a mechanically stirred mixture of 200 grams of iron filings, 400 c.c. of water, and 15 c.c. of concentrated hydrochloric acid (compare Morgan, J., 1900, **77**, 1204), the heat of reaction, after slight preliminary warming, maintaining the temperature at 95°. After an hour's heating at 90°, the whole was cooled, neutralised with 30 grams of sodium bicarbonate, and the chloroaniline distilled

in steam and obtained in 50 per cent. yield from the dried, ethereal extract of the distillate. Its solution was diazotised (Varnholt, *J. pr. Chem.*, 1887, **36**, 27), heated, filtered, and the chlorophenol extracted with ether and purified by distillation.

m-Bromophenol, prepared in a similar manner from *m*-bromonitrobenzene [bromination of nitrobenzene (Wheeler and McFarland, *Amer. Chem. J.*, 1897, **19**, 366) is much more satisfactory than chlorination], distils at 125—127°/12 mm. (Diels and Bunzl, *Ber.*, 1905, **38**, 1495, give b. p. 135—140°/12 mm.).

m-Iodophenol was obtained from *m*-nitroaniline (Nölting and Stricker, *Ber.*, 1887, **20**, 3020), the intermediate *m*-iodonitrobenzene being reduced with iron and hydrochloric acid.

4-Iodoresorcinol was prepared by Stenhouse's method (*Annalen*, 1874, **171**, 311). The compulsory use of 220 grams of litharge to iodinate 20 grams of resorcinol prevents reduction of the iodoresorcinol by the hydriodic acid produced in the reaction.

Reduction of the Halogenated Phenols.—Approximately 0·6 gram of the phenol was made up to 2·5 c.c. with glacial acetic acid in a 5 c.c. stoppered measuring cylinder, 2·5 c.c. of glacial acetic acid containing 0·410 gram of hydrogen iodide per c.c. (Lapworth and Shoesmith, *loc. cit.*) were added, and the whole was well mixed and placed in a thermostat at 25°. One c.c. portions were withdrawn at definite intervals and the liberated iodine was estimated with thiosulphate. A comparison of the ease of reduction of *o*-iodophenol, *p*-iodophenol and *p*-bromophenol was thus obtained. The results are summarised in Table IV.

This method did not furnish a satisfactory reduction curve for *o*-bromophenol, but the above results having been obtained for the iodophenols and *p*-bromophenol at 25° a series of experiments at 78° completed the comparison: 10 c.c. of a standard solution of the halogenated phenol in glacial acetic acid and 10 c.c. of constant-boiling hydriodic acid were heated together in the vapour of boiling alcohol; the liberated iodine was estimated as before. The results are summarised in Table V.

In Tables IV and V, x has the same significance as before, and t is the time in minutes from the commencement of the experiment. In Table IV, w is the total weight of the phenol used, whilst in Table V it represents the weight used in each reduction.

p-Chlorophenol was the only one of the chloro-isomerides to show any reduction under the different conditions described, and this was very slight. A solution of the phenol in glacial acetic acid containing 0·37 gram of hydrogen iodide per c.c. showed a reduction of about 10 per cent. after 4 hours. Complete reduction was never observed.

TABLE IV.

	o-Iodophenol. $w =$ 0·6104 gm.	*p*-Iodophenol. $w =$ 0·6394 gm.	*p*-Bromophenol. $w =$ 0·6070 gm.
t.	*x.*	*x.*	*x.*
15	28·2	69·0	4·4
45	56·2	88·8	8·7
105	75·6	95·4	15·9
225	90·0	100·0	28·7

TABLE V.

	o-Bromophenol. $w =$ 0·1730 gm.	*p*-Bromophenol. $w =$ 0·1719 gm.
t.	*x.*	*x.*
60	18·7	40·7
120	31·7	68·4
180	39·6	83·9
240	47·6	94·2
300	53·0	100·0

The meta-halogenated phenols did not reduce in any circumstances.

4-Iodoresorcinol was completely reduced in $\frac{1}{2}$ hour at 25° in glacial acetic acid solution containing 0·40 gram of hydrogen iodide per c.c.

The authors desire to acknowledge a grant from the Earl of Moray Research Fund and also their indebtedness to Prof. Sir James Walker, F.R.S., Prof. A. Lapworth, F.R.S., and Mr. W. O. Kermack for their interest in these investigations.

EDINBURGH UNIVERSITY.

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[From the BIOCHEMICAL JOURNAL, Vol. XX, No. 3, 1926]

LXIII. STUDIES IN CARBOHYDRATE METABOLISM.

I. THE UTILISATION OF DIHYDROXYACETONE BY THE ANIMAL BODY AND A METHOD FOR ITS ESTIMATION.

By WILLIAM OGILVY KERMACK,
CHARLES GEORGE LAMBIE (*Beit Memorial Research Fellow*),
and ROBERT HENRY SLATER.

From the Departments of Therapeutics and Pharmacology, Edinburgh University, and the Research Laboratory of the Royal College of Physicians, Edinburgh.

(*Received March 11th, 1926.*)

The possible use of dihydroxyacetone in the treatment of diabetes was suggested, apparently first of all, by Emil Fischer, and this has been followed by observations upon its clinical use by various workers, particularly in Germany [Isaac and Adler, 1924], and in Canada. Rabinowitch [1925], for example, has shown that it possesses considerable antiketogenic power and has employed it successfully in the treatment of diabetic coma.

For a long time discussion has centred around the claims of three compounds containing three carbon atoms as possible intermediaries in carbohydrate metabolism: methylglyoxal, glyceric aldehyde and dihydroxyacetone. It is impossible to discuss in detail here the respective claims of each of these, but reference may be made to the discussion by Dakin [1922].

In this connection the rather interesting fact may, however, be mentioned that dihydroxyacetone, when perfused through the liver [Embden, Baldes and Schmitz, 1912], can give rise to small amounts of *d*-lactic acid and to *d*-glucose, whereas glyceric aldehyde, under the same conditions, gives rise to large amounts of inactive lactic acid containing excess of the *l*-isomeride, and to *d*-sorbose, both of which are foreign to the animal body. In addition, glyceric aldehyde has been found [Sansum and Woodyatt, 1916] to damage the kidneys of rabbits, whereas dihydroxyacetone appears to be as innocuous as glucose itself. Moreover, *dl*-glyceric aldehyde when administered to diabetics increases the glycosuria [Wells, 1920], whereas dihydroxyacetone is almost completely utilised. Again, the tolerance for glyceric aldehyde, when injected intravenously in the healthy individual, is much less than that for glucose or for dihydroxyacetone. It has also been shown [Wind, 1925] that dihydroxyacetone is oxidised in neutral phosphate solution with great ease; for example, it absorbs atmospheric oxygen about 20–30 times as rapidly as

does fructose, which again is more easily oxidised than glucose. Further, as is well known, it reduces Fehling's solution in the cold and some preliminary experiments have shown us that at p_H 7·3 its rate of oxidation by permanganate is very considerably greater than that of glucose.

It seemed to us that one possible way of distinguishing between various suggested intermediaries is provided by the consideration that any intermediary ought to be a substance which, when administered to animals suffering from convulsions and coma due to insulin hypoglycaemia, will relieve the condition. It is not, of course, suggested that the converse is the case, since the animal body may well be able to utilise certain substances, for example mannose, which presumably do not occur naturally in it.

On the other hand, a substance might prevent hypoglycaemia by being converted into glucose, and in this case one would expect that in all its reactions it would behave similarly to glucose, only showing possibly a delayed action.

In spite of these reservations it seems of great importance definitely to establish whether or not dihydroxyacetone is able to relieve the symptoms of insulin hypoglycaemia in animals.

The following experiments demonstrate quite definitely that dihydroxyacetone produces prompt and effective recovery from insulin coma and that the recovery is at least as rapid as with glucose.

The mechanism by which this recovery is brought about has occupied our attention and is very difficult to determine conclusively. It may be, as suggested above, that the dihydroxyacetone is directly oxidised, thereby providing the necessary energy for cellular metabolism, or it may be that it is very rapidly converted into glucose which then causes recovery, perhaps owing to its restoring the necessary glucose "tension" in the tissues as suggested by Noble and Macleod [1923] with reference to other substances which relieve insulin hypoglycaemia. The evidence, on the whole, we think, accords best with the first of these assumptions. This evidence, which will be discussed later, is supported by the results obtained by Rabinowitch and others, who claim that the substance is antiketogenic and utilisable by the diabetic organism, and by the experiments and clinical observations described below.

Experimental.

The dihydroxyacetone used in the following experiments is that sold by Meister, Lucius, Brüning & Co. under the name of "oxantin." The solution in water reduces Fehling's solution and alkaline picric acid solution in the cold and gives no precipitate with phloroglucinol and sulphuric acid. The latter observation indicates the absence of glyceric aldehyde. A 50 % aqueous solution does not rotate the plane of polarisation of polarised light. It leaves no ash on ignition. When mixed with phosphorus pentoxide and gently heated it yields methylglyoxal, as stated by Meisenheimer [1912] and by Fischer and Taube [1924, 1926.]

I. *Detection and estimation of dihydroxyacetone in blood.*

It appeared to us to be of importance in connection with the present work to develop a method which would enable us to detect and to estimate approximately any dihydroxyacetone present in small quantities in blood. In this way it seemed that we might be able to obtain information as to the rate at which dihydroxyacetone is utilised in the animal body. If this substance actually forms one of the intermediates in carbohydrate metabolism it might be expected that it would be utilised very rapidly. We believe that we have obtained evidence, using the method about to be described, which shows, at least, that when injected it disappears from the blood almost immediately.

Two colour tests for dihydroxyacetone are mentioned in the literature. One is the reddish brown colour given by an alkaline solution of sodium picrate in the cold, due to the reduction of the picric acid. Compared with the colour given by an equal quantity of creatinine, it is found to have only two-fifths of the intensity. A method based on this reaction would not be very sensitive.

The other reaction, which appears to be more sensitive and which therefore we have adopted as a basis of the present method, is the colour given by sulphuric acid and a phenol. A marked coloration is given by 0·10 mg. of dihydroxyacetone to which 5 cc. of sulphuric acid containing phenol have been added. Further, the colour appears to be a permanent one and it has been found possible to make up standard solutions in sealed tubes with which the tint corresponding to an unknown may be compared. It is, of course, necessary to remove all protein from the blood to be tested and also water. On this account methyl alcohol was used as a protein precipitant as this could be readily removed *in vacuo* at a temperature not exceeding 50°. The details of the method are as follows.

Blood (0·4 cc.) is thoroughly shaken up with methyl alcohol (9·6 cc.) and allowed to stand for 2 hours. The precipitated proteins are then filtered off. The filtrate (5 cc.) is placed in a comparator tube and the methyl alcohol completely removed *in vacuo* at 50°. A solution of phenol in sulphuric acid is prepared as follows. Phenol (20 g.) is warmed up with water (1 cc.) until it melts and concentrated sulphuric acid is then added slowly with cooling until the total volume is 250 cc. This solution possesses a faint pink tinge which, however, cannot be observed in the comparator tube. It appears to keep for several weeks without deterioration. This solution (5 cc.) is added to the comparator tube and the colour, which is fully developed after 2 hours at room temperature, is then compared with a series of standard solutions prepared as follows. Varying amounts of a methyl alcoholic solution of dihydroxyacetone (0·2 %) are introduced into a series of comparator tubes. The alcohol is then removed as above, and the phenol-sulphuric acid solution (5 cc.) added to each. The tubes are then hermetically sealed. The tints appear to be permanent.

In order to determine whether the method used could safely be applied to blood and also to find out whether dihydroxyacetone when added to blood

remains unchanged for a short time and is not immediately altered, the following experiment was carried out. Varying amounts of dihydroxyacetone were added to aliquot portions of human blood and the percentage then estimated colorimetrically. The results were as follows:

Amount added mg.	Amount found mg.
0·14	0·14
0·16	0·16
0·18	0·18
0·20	0·18
0·50	0·50
1·00	1·00
1·50	1·50
2·00	2·00

As in the normal blood of rabbit, man and cat a coloration is obtained corresponding to about 0·065 % of dihydroxyacetone, the tubes in the above experiment were balanced in the comparator with one prepared from 0·4 cc. of normal blood without addition of dihydroxyacetone. The nature of the substance giving this colour is being investigated.

II. *Animal experiments.*

In the following experiments rabbits were injected subcutaneously with insulin after preliminary starvation for 24 hours, and they were left untreated until complete coma had ensued and there were no signs of spontaneous recovery. As many animals recover and relapse repeatedly, it is occasionally difficult to judge when this stage has been reached, but in the majority of instances this is not so. In this respect five animals which were treated either with pyruvic acid or with lactic acid acted as controls. In only one case did spontaneous recovery take place.

The blood-sugar was determined by MacLean's method in a sample usually obtained from the ear, but, on occasions when this was impossible, by heart puncture.

The estimation of dihydroxyacetone was carried out by the method described above. In Table I we have given the actual figures obtained without subtracting the value corresponding to the colour developed in normal blood.

The following table sums up the relevant facts.

Table I.

bbit	Weight g.	Dose of insulin units per kg.	Time for coma hours	Blood-sugar in coma %	Blood-dihydroxy-acetone in coma %	Injection.	g. per kg.	Time for recovery mins.	Blood-sugar on recovery %	Blood-dihydroxy-acetone on recovery %	Remarks
1	2000	20	5·5	—	—	Dihydroxyacetone	2	10	—	—	Relapse in 2 hrs.
2	1215	5	4·0	0·054	—	,,	4·8	12	0·065	0·20	Recovery complete and permanent
3	1275	10	5·5	0·039	—	,,	0·75	5	0·042	0·07	Relapse in 2 hrs. 45 mins.
4	1167	20	5·0	0·042	0·09	,,	2·60	10	0·06	0·20	Relapse in 1 hour
5	1911	30	4·5	0·056	—	,,	0·75	10	0·025	0·10	Relapse in 3 hrs.
6	1625	10	4·7	0·022	0·05	,,	0·70	7	0·025	0·05	Recovery complete and permanent
7	(*a*) 2220	10	4·25	0·027	0·05	Glucose	0·70	7	0·056	0·05	Relapse in 120 mins.
	(*b*) ditto 6 days later	15	4·85	—	—	Dihydroxyacetone	0·70	7	—	—	Relapse in 84 mins.
8	(*a*) 1610	15	5·85	—	—	,,	0·70	6	—	—	Relapse in 34 mins.
	(*b*) 1670	15	3·33	0·032	—	Glucose	0·70	3	0·032	—	Relapse in 36 mins.
9	1400	15	4·33	0·044	—	Dihydroxyacetone	0·70	12	0·025	0·07	Relapse in 48 mins.

We have not included in the above table certain striking experiments referred to in the discussion in which recovery took place as a result of injection of dihydroxyacetone although the animals, which had previously been treated, with negative results with other substances such as sodium pyruvate or sodium lactate, were at one time in a moribund condition and in fact were kept alive by artificial respiration.

The relapses after treatment, which were frequent, are accounted for by the large doses of insulin used and were found to occur under similar conditions after treatment with glucose. No difficulty was experienced in bringing about complete and permanent recovery with a further dose of dihydroxyacetone. Altogether 12 rabbits were treated with dihydroxyacetone and recovery took place in every case except one in which a large dose of sodium citrate (10 g. in 20 cc.) had been previously injected. Death in this case was evidently due to oedema of the lungs and alkalosis.

Similar results have been obtained in an experiment in which twenty mice were used instead of rabbits. Here, dihydroxyacetone proved to be quite effective in causing rapid recovery from hypoglycaemic convulsions and coma.

These results seem to us to leave no doubt as to the power of dihydroxyacetone to remove the symptoms of insulin hypoglycaemia.

The practically equal efficiency of dihydroxyacetone and glucose is strikingly shown by Exp. 8 in which the animal received a small dose of dihydroxyacetone and relapsed in 34 minutes. Three days afterwards, after the same dose of insulin, recovery was produced by an equal dose of glucose and relapse took place after 36 minutes.

Similarly in Exp. 7, using 10 units of insulin per kg., relapse occurred with glucose in 120 minutes, whilst using 15 units of insulin it occurred in 84 minutes after a similar dose of dihydroxyacetone.

III. *Comparison between rate of utilisation of glucose and of dihydroxyacetone by muscle.*

It has been shown elsewhere [Lambie, 1926] that when the liver is excluded from the circulation of a decerebrated and eviscerated cat, there occurs a sharp fall in the concentration of the sugar in the circulating blood, and that this fall may be counterbalanced, and the blood-sugar level kept approximately constant, by the continuous injection of glucose at a uniform rate of about 0·15 g. per kg. per hour. In the present experiment the technique described fully in the above-mentioned communication was used. A cat was decerebrated after brief etherisation and artificial respiration applied. Cannulae were inserted into each jugular vein and into one carotid artery. After rapid evisceration, the portal and renal vessels were ligated so as to exclude the liver and kidneys. Glucose transfusion was begun into one of the jugulars, while samples of blood were taken from the carotid cannula, 10, 25, and 45 minutes after commencing the injection. As shown in Table II, the blood-

sugar remained at an approximately constant level. The glucose transfusion was then stopped and immediately dihydroxyacetone was perfused at the same uniform rate through the other jugular cannula. Another sample of blood was taken 3 to 4 minutes after the change-over and further samples 15 and 35 minutes later. During the period with dihydroxyacetone a fall was observed in the blood-sugar level. At the same time dihydroxyacetone determinations showed that no significant change had taken place in the concentration of this substance in the blood. It is clear that the dihydroxyacetone injected was utilised almost immediately and at a much greater rate than glucose. It may be mentioned that experiments with other sugars, for example laevulose, show that these are not utilised at a greater rate than glucose. It is also evident that during the period with dihydroxyacetone, the total utilisation of carbohydrate is increased, for not only is the dihydroxyacetone completely used up but also a considerable amount of glucose disappears. This result cannot be explained on the assumption that dihydroxyacetone was first converted into glucose because in that case no fall in the total glucose in the blood would occur.

Table II. *A decerebrated and eviscerated cat was used; liver excluded from circulation and renal vessels ligated.*

Time					
3.0	Glucose transfusion. 0·15 g. per kg. per hour				
3.10	Blood 1	Glucose	0·357 %	Dihydroxyacetone	0·065 %
3.25	„ 2	„	0·354	„	0·065
3.45	„ 3	„	0·360	„	0·065
	Glucose transfusion stopped and dihydroxyacetone 0·15 g. per kg. per hour transfused				
3.50	Blood 4	Glucose	0·355 %	Dihydroxyacetone	0·065 %
4.5	„ 5	„	0·340	„	0·055
4.25	„ 6	„	0·299	„	0·075

Observations on man.

In order to elucidate the above results the following experiments were performed on man. In general they are confirmatory of those of Rabinowitch. In the first place estimations were made of the sugar and dihydroxyacetone in the blood of a normal individual after ingestion of 50 g. of dihydroxyacetone by the mouth. The blood-sugar curve is given in Table IV. For comparison, the curve obtained in the same individual after taking 50 g. of glucose is also given in Table III. Only a very slight rise in the reducing power of the blood is observed after dihydroxyacetone and this may well be accounted for by the formation of small amounts of hexoses in the alkaline secretion of the intestine, a suggestion put forward by Rabinowitch who, however, definitely attributed the rise to glucose. It is difficult, however, to exclude the possibility that other hexoses may be formed under these conditions, and the slight increase in the reducing power of the urine after ingestion of dihydroxyacetone may be partly due to the presence of such hexoses.

Table V indicates the blood-sugar curve of a diabetic man after ingestion of 50 g. of dihydroxyacetone. The rise in blood-sugar is much smaller than

would be observed after taking a similar amount of glucose and it is quite clear from this curve and similar ones given by Rabinowitch that rapid conversion into glucose in the blood-stream or in the tissues and organs does not take place. The small rise may well be due, again, to formation of hexoses in the intestine and, naturally, since this subject was diabetic, the resulting rise in the blood-sugar is rather greater than in a normal individual.

Table III. *Curves of sugar and dihydroxyacetone in blood of normal man after ingestion of* 50 *g. of glucose.*

Time	Blood-sugar %	Blood-dihydroxy-acetone %
9.55 a.m. (before glucose)	0·110	0·06
10.0 a.m. (glucose taken 50 g.)	0·160	0·065
10.30 a.m.	0·160	0·065
11.0 a.m.	0·126	0·060
11.30 a.m.	0·094	0·060
12.0 noon	0·082	0·060
12.30 p.m.	0·091	0·065

Table IV. *Curves of sugar and dihydroxyacetone in blood and reducing power of urine, after ingestion of* 50 *g. of dihydroxyacetone by a normal man.*

Time	Blood-sugar %	Blood-dihydroxy-acetone %	Urine sugar %
9.55	0·106	0·065	0·048
10·0	(50 g. dihydroxyacetone)		
10.30	0·114	0·070	0·132
11.0	0·098	0·075	0·171
11.30	0·102	0·085	0·145
12.0	0·106	0·075	?
12.30	0·111	0·080	0·145

The reducing power of the urine was determined by Benedict and Osterberg's method for sugar in normal urine.

The urine, after dihydroxyacetone, gave no reduction with Fehling's solution in the cold, but slight reduction occurred on heating.

Table V. *Curve of blood sugar in diabetic after* 50 *g. dihydroxyacetone by mouth.*

Time	Blood-sugar %
9.55	0·183
10.0	50 g. dihydroxyacetone
10.30	0·218
11.0	0·180
11.30	0·175
12.0	0·180

A further indication that dihydroxyacetone is not converted rapidly into glucose by the tissues is furnished by the fact that this subject, after administration of dihydroxyacetone, excreted no sugar detectable by Fehling's test in the 24 hours' urine.

Another diabetic gave the following results.

A. B., a pregnant female diabetic on a constant diet of 2000 calories and 20 units of insulin, was excreting 1 g. glucose per diem and acetone. With 15 units insulin she excreted approximately 10 g. glucose. With 20 units insulin plus 30 g. dihydroxyacetone the urine contained no acetone, and the sugar excretion did not exceed 2 g. Further investigations are being carried out on the utilisation and antiketogenic action of dihydroxyacetone from a quantitative point of view. Owing to the fluctuation in tolerance of individuals it is very difficult to obtain conclusive results.

Discussion.

In reviewing the results of the above experiments, it is convenient to consider each group separately in the first place and to discuss the possible explanations of the phenomena observed. In the case of the experiments on rabbits and mice, three possible explanations of the recovery from hypoglycaemic coma under dihydroxyacetone suggest themselves. The dihydroxyacetone might be condensed into glucose in the animal body and the glucose so formed would relieve the symptoms just as glucose does when injected as such. This view is made feasible by the observation that dihydroxyacetone is converted into glucose when perfused through the glycogen-poor liver [Embden, Schmitz, and Wittenberg, 1914], and that it causes an increased excretion of glucose in the phloridzinised dog [Ringer and Frankel, 1914]. Moreover, it has been suggested by Macleod that those sugars relieve insulin hypoglycaemia which are readily convertible into glucose, *e.g.* mannose, fructose, and possibly maltose.

There are, however, several difficulties to be considered with regard to this view. In the first place, the action of dihydroxyacetone on hypoglycaemic rabbits and mice appears to be as rapid as that of glucose and the quantities required to relieve the symptoms are approximately the same, namely 0·7 g. per kg. under the conditions of the above experiments.

If, then, this explanation were the correct one, it would imply an almost quantitative and very rapid conversion of dihydroxyacetone into glucose by the animal organism. This would mean that the blood-sugar would always be raised as it is after an injection of glucose. In three cases, however (Exps. 5, 6 and 9), it may be observed that recovery occurred when the blood-sugar was as low as 0·025 %, whereas, after the administration of glucose the blood-sugar is practically always at or above the convulsion-level of 0·04 %. Only in one case was it below this, namely in Exp. 8, when the reading of blood-sugar was 0·032 %. Naturally the variability of the convulsion-level in different animals and the experimental error in the sugar determinations have to be taken into account. In some cases where very large doses of dihydroxyacetone had been injected the apparent blood-sugar level rose to 0·065 %. In these cases, however, a correction has to be made as the concentration of dihydroxyacetone present in the blood, namely 0·135 %, which is the 0·2 %

found, minus 0·065, is itself sufficient to cause considerable reduction. In fact, it has been found that this concentration of dihydroxyacetone gives, by MacLean's method, reductions corresponding roughly to 0·03 % of glucose. When this is subtracted from the value obtained (0·06 %), the actual concentration of glucose in the blood is found to be 0·035 % and therefore no great emphasis can be laid on the apparent high value obtained.

It appears, therefore, that recovery with dihydroxyacetone usually occurs when the blood-sugar is at a lower level than that at which convulsions occur or that at which recovery takes place after the administration of glucose.

The next explanation that may be offered of the action of dihydroxyacetone is that it may be used directly. This possibility is particularly attractive in view of the experiments of Wind [1925], who has demonstrated the remarkable ease with which dihydroxyacetone is oxidised in neutral phosphate solution by gaseous oxygen, and this marked tendency to be oxidised is also illustrated by the rapid reduction of permanganate at 37° and p_H 7·3.

The remaining possibility is that dihydroxyacetone is condensed or otherwise changed into some particularly reactive sugar, which is also formed from glucose in the ordinary course of metabolism. It is, of course, difficult to exclude this explanation, but until definite evidence for the existence of such a compound is forthcoming it seems best to enquire how far the simpler hypothesis of direct oxidation will explain the facts.

The experiment on the cat shows how dihydroxyacetone is immediately removed from the circulating blood, and this at once excludes the hypothesis that dihydroxyacetone is rapidly converted into glucose in such a preparation, otherwise the blood sugar would remain level. Even the possibility that it might be converted into glucose in the cells seems improbable, otherwise the blood-sugar would remain level in the above experiments.

Taking this experiment alone, the disappearance of dihydroxyacetone might be explained as being due merely to its rapid diffusion into cells without utilisation; but this would not, of course, explain the permanent recovery obtained with rabbits and mice in which direct utilisation or conversion into glucose must take place. Here again, the hypothesis that it is rapidly oxidised seems the most feasible.

The experiments on man appear to show clearly that in the intact individual dihydroxyacetone is not rapidly and quantitatively converted into glucose. If it were, one would expect the blood-sugar curve in the normal individual after ingestion of 50 g. of dihydroxyacetone to be very similar to that after an equal amount of glucose. Again, if it were converted into glucose it should cause a marked excretion of sugar and a marked rise in the concentration of sugar in the blood in diabetes. Such a result could of course be explained on the assumption that the liver can store dihydroxyacetone more readily than it can glucose. But this hypothesis does not explain the results of the experiment with the cat in which the liver was excluded from the circulation. The possibility cannot, however, be excluded that, particularly

in a normal individual, rapid storage may take place. But it is difficult to agree that this would occur readily in the diabetic, as the formation of glucose would be the first step in the synthesis of glycogen, and glucose does not form glycogen in the diabetic.

On reviewing all the results, it is seen that although special explanations might apply to each separate set of experiments, yet all the phenomena are readily explained on the simple assumption that dihydroxyacetone is very easily and possibly directly oxidised and utilised by the animal organism. This simple hypothesis, we think, explains all the facts.

Any dogmatic conclusion would at present be premature. Further experiments with other intermediary metabolites might confirm the rough hypothesis above formulated and these are being carried out together with quantitative observations upon the utilisation and antiketogenic power of dihydroxyacetone.

In conclusion, reference may be made to a paper which has recently appeared and which has just come to our notice. In this paper Campbell [1926] describes a method for the estimation of dihydroxyacetone in blood and applies the method to the determination of dihydroxyacetone in the blood after the ingestion of 100 g. of the substance. During the first half hour a rapid rise in the blood-dihydroxyacetone from 0·0 to 0·07 % was found and this was followed by a rapid drop. The blood-sugar simultaneously rose during the first half hour but after that it usually showed a definite fall. This seems to support the view that the disappearance of dihydroxyacetone from the blood is not due to its conversion into glucose, as in that case one would expect some indication of a rise in the glucose concentration rather than a fall. The fact that Campbell does obtain a rise in the blood-dihydroxyacetone after the ingestion of dihydroxyacetone is not necessarily inconsistent with our results as Campbell used double the quantity, namely 100 g. as compared with the 50 g. that we have used. In some of our experiments with rabbits, where very large quantities of dihydroxyacetone were injected, a marked rise occurred in the concentration of the substance in the blood. Naturally, although dihydroxyacetone is utilised rapidly by the tissues, there is a limit to the rate at which utilisation can take place. The very small rises in blood-sugar which Campbell obtained after ingestion of 100 g. glucose are worthy of remark.

Summary.

1. Dihydroxyacetone is able to cause recovery of rabbits and mice from the symptoms of insulin hypoglycaemia, the amount and time required for its action being approximately the same as in the case of glucose.

2. Dihydroxyacetone is more rapidly removed from the blood stream by the muscles than is glucose or laevulose, and unless excessive amounts are given the removal is practically complete.

3. This apparently ready utilisation of dihydroxyacetone by the tissues is also observed in certain diabetic individuals.

4. The facts accord with the hypothesis that dihydroxyacetone is directly utilisable by the normal animal organism and need not first be converted into glucose.

5. A method is given for the detection and estimation of dihydroxyacetone in small amounts of blood.

We desire to express our thanks to Mr W. Leiper for carrying out the blood sugar determinations in connection with this research and to the Department of Scientific and Industrial Research for a personal grant to one of us (R. H. S.).

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VII. STUDIES IN CARBOHYDRATE METABOLISM.

II. INFLUENCE OF METHYLGLYOXAL AND OTHER POSSIBLE INTERMEDIARIES UPON INSULIN HYPOGLYCAEMIA.

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In a previous communication [Kermack, Lambie and Slater, 1926], it has been shown that dihydroxyacetone when administered subcutaneously causes rabbits and mice to recover from insulin hypoglycaemia. It seemed to us that no significant difference could be discovered between the time taken for recovery after injection of dihydroxyacetone as compared with that taken after injection of glucose. In a paper by Campbell and Hepburn [1926], it has likewise been demonstrated that dihydroxyacetone causes prompt recovery from the symptoms of insulin poisoning, but these workers are of the opinion that glucose effects recovery more rapidly than does dihydroxyacetone, at least when both substances are injected intravenously. On the other hand, Hewitt and Reeves [1926], who have also confirmed the fact that dihydroxyacetone cures insulin hypoglycaemia, state that after administration of dihydroxyacetone recovery takes place in a manner identical with that seen when glucose is injected. They, like ourselves, injected the carbohydrate subcutaneously. We have now carried out further experiments to discover whether the discrepancy between our results together with those of Hewitt and Reeves on the one hand, and those of Campbell and Hepburn on the other, was due to the method of administration. We have not been able to convince ourselves that with intravenous injection there is any significant difference in the time taken for recovery. Naturally, without using very large numbers of animals it would be impossible to determine this point conclusively owing to the varying response of different animals or even of the same animal at different times.

In the paper by Hewitt and Reeves, referred to above, it is shown that glyceric aldehyde is not able to effect recovery from insulin hypoglycaemia.

We have prepared methylglyoxal by a method described by Fischer and Taube [1924, 1926] and we have tested this substance in a similar way. Briefly, we may say that the results were entirely negative and we even

received the impression that the substance possessed some toxicity. In all, seven animals were tested and in none of them did recovery take place. In each case the substance was injected subcutaneously, and usually several doses were administered. The rabbits were allowed to become completely comatose before injection of the test substance in order to minimise the risk of fallacies due to spontaneous recovery.

The following is a typical experiment.

Rabbit, female, weight 2320 g., starved 24 hours:

12.0 noon. 10 units insulin per kg.
2.50 p.m. Convulsions followed by recovery.
3.45 ,, Convulsions with imperfect recovery.
4.0 ,, Convulsions and coma.
4.18 ,, Methylglyoxal 0·7 g. per kg. subcutaneously.
4.21 ,, Violent convulsions.
4.32 ,, Sprawling.
4.41 ,, Convulsion, deeply comatose.
4.44 ,, 0·7 g. methylglyoxal per kg.
4.48 ,, Deeply comatose, lying on side apparently moribund, rapid respiration.
4.55 ,, Violent convulsion.
5.5 ,, Dihydroxyacetone 0·7 g. per kg. subcutaneously.
5.10 ,, Sitting up in normal position.

Recovery complete and permanent.

Three of the animals to which large quantities of methylglyoxal were given, failed to recover even after subsequent injection of glucose or dihydroxyacetone. It is because of this and the peculiar deep breathing observed in these hypoglycaemic rabbits after repeated large doses of methylglyoxal, that we are led to believe that it possesses some toxic action.

A note may be added here on the preparation of methylglyoxal from dihydroxyacetone.

Experiments carried out according to the instructions of Fischer and Taube in which dihydroxyacetone (5·0 g.) and phosphorus pentoxide (15·0 g.) were thoroughly mixed and very gently heated until the reaction proceeded spontaneously, the product being condensed by liquid air or by a good freezing mixture, did not give yields as good as those claimed by these authors. The product, the yield of which varied from 1–2 g., polymerised very rapidly and became insoluble in water and so had to be mixed with an equal volume of water very soon after the distillation. If the quantity of phosphorus pentoxide was reduced, the yield became greater (up to 2·6 g.), but the product obtained was less pure, as shown by the yield of di-semicarbazone (M.P. 253°), obtained from a weighed quantity.

For instance, the products from two experiments using the larger quantity of phosphorus pentoxide gave 96 % and 99 % respectively of the theoretical yield of semicarbazone, whilst with the smaller quantity only 60·5 % of the

theoretical yield was obtained. The material obtained by the use of the smaller quantity of phosphorus pentoxide did not become vitreous as the result of polymerisation as did the other, but only thickened to a very viscous consistency.

Since either dihydroxyacetone or methylglyoxal might conceivably form lactic acid in the animal body, while the latter might either undergo complete combustion or form glucose, it was considered of interest to investigate further the influence of this acid upon insulin hypoglycaemia. We entirely failed to effect recovery or to prevent convulsions, either by subcutaneous or by intravenous injection of the substance. Our experiments therefore confirm the impression gained by Noble and Macleod [1923] who, while not coming to any final conclusion, did not obtain evidence of recovery.

Glycerol also failed to produce recovery or to prevent convulsions, even when large quantities were injected intravenously. This agrees with the results of Noble and Macleod [1923], although it should be noted that Voegtlin, Dunn and Thompson [1924, 1925], claim that glycerol administered intraperitoneally or by mouth protects mice from insulin hypoglycaemia and causes a rise in the blood-sugar of fasting rabbits. If it is active at all, it is certainly, in our experience, in no way comparable to either dihydroxyacetone or glucose.

Sodium pyruvate, like methylglyoxal, so far from removing the symptoms of hypoglycaemia, appeared to have a distinct toxic action, which, in some animals interfered with the recovery by glucose or dihydroxyacetone.

During the present work, sodium citrate and rhamnose were also tested, in each case with negative results.

Discussion.

From the above, it is evident that there can be no comparison between dihydroxyacetone and any of the other assumed intermediaries in carbohydrate metabolism containing three carbon atoms, namely, methylglyoxal, glyceric aldehyde, glycerol, lactic acid and sodium pyruvate.

In our previous communication we left it an open question as to whether dihydroxyacetone acted by being converted into glucose or whether it could be directly oxidised, although we came to the conclusion that the balance of evidence was in favour of the latter assumption. Since this communication was written, a paper has appeared by Campbell, Fletcher, Hepburn and Markowitz [1926] in which the conclusion is reached, from a number of different lines of investigation, that dihydroxyacetone is apparently completely converted into glucose as the first step in its metabolism in the animal body. Nevertheless, we consider that such a definite conclusion is not justified. There is, we think, a considerable body of evidence to show that dihydroxyacetone may be metabolised more rapidly than glucose. Firstly, the blood-sugar curves reported by Isaac and Adler [1924], Rabinowitch [1925, 1, 2], Mason [1926, 1] and ourselves all indicate that dihydroxyacetone does not

cause an increase in the blood-sugar comparable to that produced by an equal amount of glucose. Even Campbell [1926] found very little rise in the blood-sugar curve after dihydroxyacetone although, curiously, he got very little rise in his control experiments with glucose. Secondly, Isaac and Adler [1924] have brought evidence to show that dihydroxyacetone can form glycogen with greater ease than can glucose. Thirdly, the respiratory metabolism experiments of Mason [1926, 2] indicate that dihydroxyacetone is oxidised more easily and more completely than is glucose in the normal individual and to a less extent in the diabetic[1]. Fourthly, in the perfusion experiment upon the eviscerated and decerebrate cat reported in our first communication, it was shown that dihydroxyacetone disappears from the blood more rapidly than glucose when perfused at the same rate, even although the liver is out of the circulation.

The problem is a difficult one, but it is not easy to reconcile these observations with the conclusions reached by Campbell, Fletcher, Hepburn and Markowitz. Particularly when we consider the greater ease with which dihydroxyacetone can be oxidised *in vitro* as compared with glucose does it seem probable that it can undergo, to some extent at least, direct oxidation. Campbell, Fletcher *et al.* found that in the completely depancreatised dog dihydroxyacetone was not only converted into glucose and quantitatively excreted as such, but that the elimination was as rapid as if an equivalent quantity of glucose had been administered. We have examined two cases of complete diabetes in the human subject, that is to say, patients who were excreting more sugar than they were taking in. In such clinical experiments it is difficult to obtain clear cut results but in general our conclusion was that the dihydroxyacetone administered was almost quantitatively excreted as glucose. Incidentally, it was confirmed by polariscopic examination that the reducing substance in the urine was actually dextrose.

It seems then that in extreme diabetes dihydroxyacetone may be converted rapidly into glucose, whereas, where insulin is available, it is more easily utilised than glucose. This conclusion is confirmed by the observations of Mason [1926, 2], who found that in diabetics dihydroxyacetone did not exhibit as great a difference in its rate of metabolism when compared with glucose as was found in the case of normal individuals.

In order to explain this result we venture to suggest the hypothesis that an equilibrium exists in the body between dihydroxyacetone and glucose (similar to that which appears to exist *in vitro* in weak alkaline solution), such that in diabetes or in the absence of insulin the tendency is in the direction dihydroxyacetone to glucose and in the opposite direction when there are inadequate supplies of insulin. Administration of excess of dihydroxy-

[1] [Note added January 1, 1927.] Since the present paper was submitted for publication, the results of Mason have been amply confirmed by one of us (C. G. L.) and it has further been shown, by intravenous injection, that greater and more rapid increase in metabolism after dihydroxyacetone, as compared with glucose, is not due to its more rapid absorption. These results will be published fully elsewhere.

acetone, even in the presence of insulin, would by mass action give rise to a transient hyperglycaemia.

Dihydroxyacetone will naturally be in equilibrium with glycerol and with methylglyoxal, and through the latter with lactic acid and alanine. If dihydroxyacetone is being rapidly converted into glucose, as in the diabetic organism, not only will all these substances tend to produce glucose, but also compounds such as propionic acid and fatty acids containing an odd number of carbon atoms as well as certain amino-acids which ultimately on degradation give rise to fragments containing three carbon atoms. On the other hand, after administration of dihydroxyacetone, an increase in the excretion of lactic acid is to be expected, as this is a comparatively stable product in equilibrium with dihydroxyacetone through methylglyoxal, and it has been shown, in fact, by Isaac and Adler [1924] and by Mason [1926, 1, 2] that there is an increase in the production of lactic acid under these conditions. Again, it has been shown by Smedley MacLean and Hoffert [1926] that in yeast the probability is that fat is produced directly from at least three glucose molecules and, if this applies to the animal organism, the tendency often observed in early diabetics to the deposition of fat would be simply due to the mass effect of the large amount of glucose formed and its non-conversion into dihydroxyacetone. Naturally, the fat deposits disappear when the organism becomes increasingly dependent upon these stores of fat as a source of energy. The glycerol formed from their catabolism would then go through dihydroxyacetone into glucose and, of course, it is well known that glycerol yields glucose quantitatively in the complete diabetic.

It at first seems an important objection to this view that dihydroxyacetone exists, if it exists at all, in the blood stream in a concentration of at any rate less than 1 in 20,000 (human), to 1 in 10,000 (rabbit). However, if dihydroxyacetone is extraordinarily easily oxidised and if the conversion of glucose into dihydroxyacetone under the influence of insulin only takes place comparatively slowly as compared with the rate of oxidation of dihydroxyacetone, and further when it is remembered that the site of the action of insulin is not in the blood but in the tissues [Eadie, Macleod and Noble, 1923; Burn and Dale, 1925; Lambie, 1926], such a low concentration of dihydroxyacetone in the blood stream is only to be expected.

If the above hypothesis were true, the effect of dihydroxyacetone in causing animals to recover from insulin hypoglycaemia would be that it is directly oxidised, thereby supplying energy for the activity of the nerve cells. The absence of effect in the case of any of the other supposed intermediaries would be due to the fact that they can neither be themselves oxidised in such a way that their energy can be utilised in cellular metabolism nor converted into dihydroxyacetone at a sufficient rate. Presumably the only substances which could be so converted would be glucose, fructose and the few substances which cause recovery from insulin hypoglycaemia.

That glucose and dihydroxyacetone appear to effect recovery in the same

time may be due to the fact that the limiting factor is not simply the rate at which a sufficient quantity of dihydroxyacetone is brought in contact with the tissues, whether from glucose or directly, but the time taken for the nerve and muscle cells to recover from the secondary effects of the hypoglycaemic state.

SUMMARY.

(1) No significant difference could be observed in the time taken to recover from insulin hypoglycaemia following intravenous dihydroxyacetone administration as compared with glucose.

(2) Methylglyoxal and sodium pyruvate fail to cause animals to recover from insulin hypoglycaemia and appear to have a toxic action.

(3) Negative results were also obtained with sodium lactate, glycerol, sodium citrate and rhamnose.

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XLVII. STUDIES IN CARBOHYDRATE METABOLISM.

IV. ACTION OF HYDROXYMETHYLGLYOXAL UPON NORMAL AND HYPOGLYCAEMIC ANIMALS.

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Three compounds containing three carbon atoms have frequently been considered as possible intermediaries in carbohydrate metabolism: glyceraldehyde, dihydroxyacetone, and methylglyoxal. If the formula of hydroxymethylglyoxal, $CH_2OH.CO.CHO$, is compared with those of these three compounds it is seen that it bears a close relationship to all of them, and may, in fact, be regarded as an oxidation product of any of them. It was therefore considered of some interest to ascertain the action of this compound on the animal organism. This appeared the more desirable since evidence has been advanced by Hynd [1927] and Herring and Hynd [1928] which, it is claimed, supports the view that glucosone, $CH_2OH.(CH.OH)_3.CO.CHO$, is amongst the products formed in the animal body by the oxidation of dextrose under the action of insulin. In particular, they have found that the administration of glucosone to various animals causes the appearance of symptoms similar to those associated with insulin hypoglycaemia in the same species. The suggestion is that glucosone is the compound directly responsible for the appearance of these symptoms. Hydroxymethylglyoxal may be regarded as the simplest compound analogous to glucosone, bearing in fact the same relation to the triose glyceraldehyde that glucosone bears to dextrose. It was therefore of interest to determine whether hydroxymethylglyoxal causes the appearance of toxic symptoms similar to those produced by glucosone.

It may be noted that White and Willaman [1928] have found that hydroxymethylglyoxal is readily fermented by *Fusarium lini*, the only other compounds showing an equal ease of degradation by this mould being dihydroxyacetone and the fermentable hexoses.

Experimental.

The hydroxymethylglyoxal used in the following experiments was prepared by the method of Evans and Waring [1926], with one slight modification. These authors obtained good yields of the compound by oxidising dihydroxyacetone with copper acetate, allowing the mixture to stand for 10 days at ordinary laboratory temperature. We have been unable to obtain a crystalline product under these conditions, but found that the syrup produced rapidly crystallised if the oxidation had been allowed to continue for 30 days at room temperature or for 25 days at 25°. This difference is probably due to the fact that the laboratory temperature was considerably below that which prevails in American laboratories. With this modification we found that the reaction proceeds and the product crystallises just as described by Evans and Waring. It may be mentioned here that these authors found that when solid hydroxymethylglyoxal is dissolved in cold water it exists in a dimeric form, but when heated for 5 minutes at 100° it changes into the monomeric state.

Effect of hydroxymethylglyoxal on normal mice.

Experiments were first of all carried out to ascertain the effect of hydroxymethylglyoxal on normal mice, first with a freshly prepared solution of hydroxymethylglyoxal which had not been heated. The results are summarised in the following table.

Table I. *Effect of injections of unheated hydroxymethylglyoxal solution on mice.*

No. of exp.	Colour of mouse	Vol. of solution injected cc.	Concn. of hydroxy-methyl-glyoxal %	Wt. of hydroxy-methyl-glyoxal g.	Remarks
1	Albino	0·4	15	0·06	Sprawling in 1 minute then lying on side comatose. Breathing shallow, then gasping. Death in 3½ minutes
2	Albino	0·2	15	0·03	Arrest of respirations, convulsions and death in 2 minutes
3	Albino	0·4	7·5	0·03	Convulsions and death in 3 minutes
4	Black and white	0·3	7·5	0·0225	Convulsions, coma and death in 3 minutes
5	Black and white	0·2	7·5	0·015	Drowsiness, tail erected, paddling movements, coma, followed by recovery
6	Black	0·2	7·5	0·015	Erection of tail, convulsions, running movements, coma and death in 3 minutes
7	Black and white	0·2	7·5	0·015	Drowsiness, sprawling, paddling movements. Recovery in 2 hours
8	Albino	0·2	7·5	0·015	Convulsions, coma and death in 4 minutes
9	Black	0·2	7·5	0·015	Convulsions, coma and death in 4 minutes
10	Black	0·2	7·5	0·015	Drowsiness and sprawling, no convulsions. Recovery
11	Black	0·2	7·5	0·015	Drowsiness and sprawling, no convulsions. Recovery
12	Black and white	0·4	0·75	0·003	No symptoms

It will be seen that the lethal dose for a mouse is approximately 0·015 g. In those experiments in which death did not occur rapidly the animals exhibited a train of symptoms resembling those resulting from insulin hypoglycaemia. Thus in mouse No. 5, after a preliminary period during which the respirations were accelerated, the animal became drowsy for a time, then later the respirations became shallow and irregular, the tail was erected and the animal showed running movements. It then became comatose and lay on its side, respiration being barely perceptible, and in fact it was to all appearances moribund. After 20 minutes however, it showed signs of recovery, the respirations increased and the animal sat up unsteadily, the legs sprawling and the tail showing the peculiar stiffening commonly noted in the mouse during insulin hypoglycaemia. It remained in a languid condition for 3 to 4 hours and ultimately recovered completely.

The second series of experiments was carried out with a solution of hydroxymethylglyoxal which had previously been heated to 100° for 10 minutes. The results are summarised in Table II.

Table II. *Effect on mice of injections of hydroxymethylglyoxal solution, heated to* 100° *for* 10 *minutes.*

No. of exp.	Colour of mouse	Vol. of solution injected cc.	Concn. of hydroxy-methyl-glyoxal %	Wt. of hydroxy-methyl-glyoxal g.	Remarks
1	Brown	0·2	7·5	0·015	No symptoms
2	Albino	0·4	7·5	0·03	No symptoms
3	Black	0·2	15	0·03	Temporary excitement. No other symptoms
4	Albino	0·2	15	0·03	Transient muscular weakness; sprawling. No other symptoms
5	Albino	0·2	15	0·03	Became inactive for a short time. No other symptoms
6	Albino	0·4	15	0·06	After 8 minutes weakness of extremities. Moved about with difficulty. No convulsions. No coma. Breathing gradually became shallower. Death in 3 hours

It will be seen that the lethal dose of monomeric hydroxymethylglyoxal is 0·06 g., which is about four times the lethal dose of the unheated material. It appears, therefore, that the depolymerisation considerably reduces the toxicity of hydroxymethylglyoxal. Further, the depolymerised substance when administered in sublethal doses does not appear to cause the appearance of symptoms similar to those of insulin hypoglycaemia.

In the preparation of hydroxymethylglyoxal, it appears very difficult to eliminate the last traces of hydrogen sulphide and colloidal sulphur. The sulphur partially separates when the solution is heated to 100°. It seems, however, that hydrogen sulphide plays no part in the production of the toxic symptoms, since no symptoms were produced in control experiments

in which much larger quantities of hydrogen sulphide were employed than were contained in the unheated solution of hydroxymethylglyoxal. Further, the very small precipitate formed by heating the solution had no toxic effects.

Effect of hydroxymethylglyoxal on hypoglycaemic mice.

Ten mice were brought into a hypoglycaemic condition as the result of the administration of 0·2 cc. of standard insulin solution (20 units per cc.), diluted 1 in 25. To five of these mice sublethal doses of an unheated solution of hydroxymethylglyoxal were administered, and to the other five sublethal doses of a solution of hydroxymethylglyoxal previously heated to 100° for 15 minutes. In no case did recovery take place, but the animals recovered promptly after the administration of glucose. Hydroxymethylglyoxal is evidently without effect on insulin hypoglycaemia. It may be added that dextrose is without effect on the toxic symptoms produced by hydroxymethylglyoxal.

Effect of hydroxymethylglyoxal on rabbits.

The above results have all been confirmed by experiments on rabbits. These animals do not recover from hypoglycaemia as the result of the administration of hydroxymethylglyoxal, but rather are made worse, and death frequently follows in 10 or 15 minutes when the unheated material is used. This fatal result was observed even with doses which, when administered subcutaneously to normal animals, caused no obvious symptoms. In this respect the result is analogous to that previously observed with methylglyoxal [Kermack, Lambie and Slater, 1927]. As much as 9 cc. of a 25 % solution of hydroxymethylglyoxal, administered subcutaneously, produced no effect even when the solution had not been heated. When unheated hydroxymethylglyoxal was administered intravenously, 0·25 g. was sufficient to cause asphyxial convulsions and cessation of respiration following a momentary stimulation of the breathing. After generalised convulsions the animal lay on its side, showing running movements and intermittent gasping respirations. Artificial respiration was performed and the animal gradually recovered. On the other hand intravenous administration of 1·25 g. of a solution of hydroxymethylglyoxal, which had previously been heated to 100° for 10 minutes, produced no toxic symptoms. In relation to the results of Hynd, which have been mentioned above, an experiment may be reported here in which enormous doses of insulin (up to 200 units), were administered to rabbits and at the same time very large quantities of dextrose, to prevent reduction in blood-sugar. As expected, no hypoglycaemic symptoms occurred. If insulin produced hypoglycaemic convulsions as the result of the conversion of dextrose into glucosone it appears probable that such symptoms would have been observed in these experiments.

Discussion.

The above experiments demonstrate the highly toxic nature of hydroxymethylglyoxal in its dimeric form. Herring and Hynd [1928] state that 2·4 mg. of glucosone per g. body weight is sublethal for a mouse. In the case of dimeric hydroxymethylglyoxal subcutaneous administration of 0·75 mg. per g. body weight is sufficient to cause death. The symptoms produced by dimeric hydroxymethylglyoxal are similar to those described by Hynd and Herring as being produced by glucosone. On the other hand, 3 mg. of the monomeric form of hydroxymethylglyoxal per g. body weight were necessary to cause death in the mouse and the animal did not exhibit the characteristic train of symptoms above noted. It is surprising to find that the monomeric form, which gives the Schiff's reaction more readily than the dimeric form, and of which the aldehyde groups are therefore in a more active condition, is the less toxic. As pointed out by Olmsted and Logan [1923], the symptoms of insulin hypoglycaemia resemble those produced by asphyxia in the rabbit and cat. It would appear that many substances which interfere with the normal oxidative processes in cells (*e.g.* such as cyanide poisoning, cf. Hess [1921], carbon monoxide poisoning, etc.), tend in the lower animals to produce a train of symptoms which have many features in common, especially within the same species. Among these symptoms are muscular weakness (as shown by sprawling of the limbs or drooping of the head), head retraction, weakness, drowsiness, convulsive seizures with paddling and running movements, and ultimately coma. In the absence of other evidence, it would appear particularly hazardous to conclude that glucosone plays a part in the production of the symptoms of insulin hypoglycaemia because of the resemblance of these symptoms to those produced by insulin. The above experiments show that almost identical effects are produced even more readily by hydroxymethylglyoxal. It is also difficult to explain the non-appearance of such symptoms after the administration of enormous doses of insulin, provided that sufficient dextrose is given to prevent a fall in blood-sugar below the normal. Reference may also be made to the fact that in cases of diabetic coma large quantities of insulin are often given without harmful effect as long as the blood-sugar is not allowed to fall unduly. Even Herring and Hynd [1928] find it necessary to suggest that the sensitiveness of the animal to glucosone depends on the blood-sugar being abnormally low; and so in the absence of independent evidence as to the existence of glucosone in the animal body it does not seem to simplify matters to assume the development of a specific poison to explain the symptoms. It must also be remembered that the symptoms of glucosone or hydroxymethylglyoxal poisoning are not relieved by the administration of dextrose.

In its inability to relieve the symptoms of insulin hypoglycaemia, hydroxymethylglyoxal resembles the majority of compounds containing three carbon atoms of which, so far, only one, namely dihydroxyacetone, has been found to possess the power of bringing about rapid recovery.

SUMMARY.

1. Hydroxymethylglyoxal in its dimeric form is highly toxic to mice and rabbits and in sublethal doses produces symptoms similar to those of insulin hypoglycaemia.

2. In its monomeric form the toxicity is reduced by 75 % and the same train of symptoms is not produced.

3. Hydroxymethylglyoxal, either in the dimeric or monomeric form, is unable to cause recovery from insulin hypoglycaemia.

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XLVIII. STUDIES IN CARBOHYDRATE METABOLISM.

V. EFFECT OF ADMINISTRATION OF DEXTROSE AND OF DIHYDROXYACETONE UPON THE GLYCOGEN CONTENT OF MUSCLE IN DEPANCREATISED CATS.

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Introduction.

It has been shown by Burn and Dale [1924] and by Lambie [1926] that administration of insulin to decerebrated and eviscerated cats results in a more rapid disappearance of dextrose from the blood stream than when no insulin is given. It has further been shown by Best, Hoet and Marks [1926] and by Best, Dale, Hoet and Marks [1926] that a large proportion of the sugar which thus disappears is laid down as glycogen in the muscles.

Much discussion has centred around the question as to whether the essential action of insulin is to convert dextrose into some intermediary substance such as dihydroxyacetone. It therefore seemed desirable to carry out experiments with a view to ascertaining whether dihydroxyacetone causes the deposition of glycogen more readily than does dextrose. Mostowski [1911] showed that the administration of dihydroxyacetone to chickens results in the deposition of glycogen in the liver, whilst in more recent years Isaac and Adler [1924] have claimed that dihydroxyacetone, when injected intraperitoneally into mice and rats, effects more rapid deposition of glycogen in the liver than does dextrose. Cori and Cori [1928] have studied the distribution of glycogen in the livers and muscles of rats fed on these sugars, and found that at the end of 4 hours the rats fed on dextrose deposited 18 % of the sugar absorbed as glycogen in the liver and 25 % as muscle-glycogen, whereas in the case of dihydroxyacetone 21 % was laid down as liver-glycogen and only 15 % as muscle-glycogen. When insulin was administered the distribution of glycogen as between liver and muscle was 6 % and 36 % for dextrose and 2 % and 33 % for dihydroxyacetone, respectively. In both cases dihydroxyacetone failed

to lay down glycogen in the muscles as readily as dextrose but in the case of the liver the percentages were approximately the same. As Cori and Cori point out, these figures cannot be taken as representing the ease of formation of glycogen, since the fraction deposited in the muscles would, other things being equal, be decreased either by increased oxidation or by increased deposition in the liver. No conclusion can therefore be drawn from these experiments as to the relative ease with which the two sugars form muscle-glycogen.

Campbell and Markowitz [1927, 1, 2] have brought forward evidence which seems to prove that in the hepatectomised animal the administration of dihydroxyacetone is unable to cause recovery from the symptoms of hypoglycaemia and that the triose is quite unattacked by the organism under these conditions. These results suggest that dihydroxyacetone is converted in the liver either into dextrose or into some active form of the triose. It is known that dihydroxyacetone can, under certain conditions, be converted by the liver into dextrose, but, whilst this would account for certain resemblances in the physiological behaviour of the two sugars, the differences which they exhibit could not be satisfactorily explained on this hypothesis alone. It therefore seemed possible that experiments on animals in which the liver was intact might afford evidence that an active substance was formed from dihydroxyacetone. The following experiments were therefore carried out upon decerebrated and depancreatised cats in which the liver remained intact.

Experimental.

Cats were employed in all experiments. After brief etherisation the brain was destroyed with a probe through a trephine opening in the skull, and artificial respiration was carried out by means of a cannula inserted into the trachea. Cannulae were also placed in the left jugular vein and the left carotid artery. After withdrawing a sample of blood for analysis (Blood 1 in table), the abdomen was opened and the pancreas was removed. In one experiment only (No. 19 in table) was the animal eviscerated and the liver and kidneys tied off, thus excluding them from the general circulation. After pancreatectomy or evisceration, as the case may be, a second sample of blood (Blood 2 in table) was taken and then transfusion of the sugar under examination was begun. The sugar (dextrose or dihydroxyacetone) was dissolved in saline in the requisite concentration and, by means of the apparatus previously described [Lambie, 1926], was run in at a constant rate through the cannula in the jugular vein. Immediately after the perfusion had begun a ligature was placed round the right femoral artery and the muscles of the right lower limb were removed, cut into small pieces with scissors, weighed *en masse*, and then dropped into boiling 60 % potassium hydroxide solution. After the transfusion had continued for a given time, a third sample of blood (Blood 3 in table) was withdrawn and the left hind limb was removed and added to potassium hydroxide solution after cutting and weighing. The

glycogen content of the muscles was then determined by means of the following method.

Estimation of glycogen in muscle.

The weighed muscle (about 100 g.) was dropped into a conical flask containing hot aqueous 60 % potassium hydroxide (100 cc.) as described above and the mixture heated in a boiling water-bath for about 6 hours when the solid material had been practically completely decomposed and a reddish brown solution remained. On cooling, this solution was poured into a standard 250 cc. flask and diluted with water to the mark. After thorough mixing 5 cc. of the solution were pipetted into a 25 cc. centrifuge tube. Absolute alcohol (20 cc.) was then added and the mixture allowed to stand for 20 hours when the glycogen was completely precipitated. The precipitated glycogen was centrifuged off and washed twice with 66 % alcohol, then with absolute alcohol and finally with ether. It was then dissolved in 2 % hydrochloric acid (10 cc.) and heated on the boiling water-bath for 4 hours. The acid solution was washed into a 25 cc. standard flask and diluted to the mark. The glucose present in a convenient volume of the solution was then estimated by the Hagedorn-Jensen method [1923] and the percentage of muscle-glycogen calculated from this result.

In certain experiments curare was employed in order to paralyse the motor nerve endings of the muscle and eliminate any muscular twitching which might alter the glycogen content of the muscles. A solution, in saline, of the residue formed by evaporation to dryness of an alcoholic extract of the bark of *Strychnos toxifera*, 1 cc. of which had in control experiments been found to paralyse completely the motor nerve endings of the muscles of the lower limb of the cat, was injected in the requisite dose immediately after decerebration had been performed and artificial respiration begun.

As it was advisable to avoid as far as possible stimulation of the pancreas, ergotamine tartrate (5 mg.) was injected 2 hours before the beginning of the experiment with a view to paralysing the terminations of the splanchnic nerves in the liver and thus limiting the initial hyperglycaemia occasioned by the destruction of the brain and the ether anaesthesia, until the pancreas could be removed. It was found, however, that the ergotamine, by paralysing the sympathetic endings in the heart, often caused excessive slowing of that organ and the resulting depression of the circulation was frequently so great that circulatory failure followed the administration of the anaesthetic. When this threatened, adrenaline, 0·2 cc. of 1 in 1000 solution, was injected intravenously in order to save the animal. The results are summarised in Table I.

The total reducing power of the blood, as given in the table, in terms of glucose, was determined by the method of Hagedorn and Jensen [1923], whilst the method of Campbell [1926] was used for the determination of dihydroxyacetone. It was found by the Hagedorn and Jensen method that 1 g. of dihydroxyacetone is equivalent to 1·48 g. of glucose by this method.

Discussion.

The single experiment in which the liver was excluded from the circulation and the animal perfused with dihydroxyacetone showed a slight fall in muscle-glycogen in spite of the fact that the blood at the end of the experiment contained 0·1 % of dihydroxyacetone. This seems to indicate, in agreement

Table I.

No. of exp.	Wt. of cat g.	Sugar perfused	Concn. of sugar in perfusion fluid %	Rate of perfusion per hour g.	Time of perfusion mins.	Total wt. of sugar perfused g.	Blood-sugar (total reducing power) mg. per 100 cc. 1	2	3	Glycogen in muscle mg. per 100 g. Before	After	Difference	Remarks
1	2600	Dextrose	40·6	2·02	90	3·04	276	369	1560	234	298	+ 64	Pancreas removed.
2	—	,,	40·0	2·0	130	4·30	222	276	318	253	201	− 52	Liver intact
3	2300	,,	34·5	1·72	117	3·44	—	—	—	476	170	−306	Curare. Pancreas removed. Liver intact
4	—	,,	30·0	1·5	175	4·50	84	106	588	359	238	−121	Ergotamine. Pancreas removed. Liver intact
5	—	,,	30·0	1·5	100	2·50	282	438	—	307	455	+148	
6	—	,,	30·0	1·5	135	3·37	254	326	580	574	562	− 12	Ergotamine. Adrenaline. Pancreas removed. Liver intact
7	—	,,	60·0	6·25	106	11·04	144	198	2220	279	206	− 73	
8	—	,,	30·0	1·5	130	3·25	—	104	456	658	687	+ 29	
9	2300	Dihydroxyacetone	30·0	1·5	120	3·00	258	326	870	402	407	+ 5	
10	3200	,,	50·0	2·5	70	2·91	—	318	1560 (0·42 % Di.)	194	242	+ 48	
11	2250	,,	50·0	2·5	90	3·75	140	158	1140 (0·1 % Di.)	592	421	−171	Pancreas removed. Liver intact
12	2270	,,	35·4	1·77	90	2·66	222	343	1020 (0·173 % Di.)	274	288	+ 14	
13	—	,,	40·0	2·0	80	2·66	258	404	1230	149	206	+ 57	
14	—	,,	40·0	2·0	110	3·66	154	220	1710	381	372	− 9	
15	2100	,,	31·5	1·58	120	3·15	—	—	—	274	223	− 51	Curare. Pancreas removed. Liver intact
16	2400	,,	37·4	1·87	90	2·80	—	—	—	499	577	+ 78	
17	—	,,	30·0	1·5	80	2·0	230	426	1008	559	743	+184	Ergotamine. Adrenaline. Pancreas removed. Liver intact
18	—	,,	60·0	6·25	90	9·37	272	282	3180	989	564	−425	
19	3450	,,	53·0	2·69	90	4·03	—	254	1140 (0·1 % Di.)	252	232	− 20	Complete evisceration. Liver tied off

The calculated Blood 3 glucose values in experiments Nos. 10, 11, 12 and 19 are 938, 992, 764 and 992 mg. per 100 cc. respectively.

with the conclusion of Campbell and Markowitz [1927, 1, 2], that glycogen is not produced readily from dihydroxyacetone in the absence of the liver, but further experiments are necessary to settle this point.

It will be noted that in certain experiments ergotamine or curare was administered to the animal. As explained above, this was done with the aim of preventing glycogenolysis in the liver or muscles respectively but there was no evidence that the curarised animals gave more constant results than the untreated ones, whilst the animals which had been treated with ergotamine frequently suffered from circulatory failure and usually died if not given adrenaline. Moreover the ergotamine was frequently ineffective in paralysing the splanchnics completely.

It is evident from the figures in the preceding table that no significant difference is observable under the above conditions in the power of dextrose and of dihydroxyacetone to cause deposition of glycogen in the muscles. In the eight experiments with dextrose the mean decrease in muscle-glycogen from the beginning to the end of the perfusion period was $0{\cdot}040 \pm 0{\cdot}051$ %, whilst when dihydroxyacetone was used the decrease was $0{\cdot}027 \pm 0{\cdot}054$ %. It is obvious, however, that in individual experiments a change in the glycogen is quite considerable and the question arises as to whether this can be correlated with any of the other factors which vary from experiment to experiment.

Inspection shows that with the exception of experiment No. 18, a marked fall in glycogen is usually associated with a lower initial blood-sugar, whilst a marked rise in glycogen is usually observed with a high initial blood-sugar. The figures (including Exp. 18) were therefore examined statistically, taking the 13 experiments in which the initial blood-sugar had been determined, in order to find out whether any correlation existed between the level of initial blood-sugar and the change in the muscle-glycogen. The value of the partial correlation coefficient between initial blood-sugar level and change in muscle-glycogen for a constant initial glycogen content was found to be $-0{\cdot}30 \pm 0{\cdot}15$. This correlation coefficient would have been considerably greater but for experiment No. 18. It may be noted that in this experiment an unusually high initial muscle-glycogen existed and the large fall in glycogen content may be related to this factor. Because of the comparatively small number of animals (13), the probable error of the correlation coefficient is high and too much emphasis must not be laid on the result, but the above experiments suggest that the level of the initial blood-sugar in such a perfusion probably plays a rôle in determining the alteration in glycogen. This may be due to the stimulation of the pancreas, in the short time that elapses before it is excised, so that it secretes insulin and in this way brings about the deposition of glycogen.

Whilst these experiments do not prove conclusively that dihydroxyacetone cannot cause deposition of glycogen in the muscles more readily than dextrose, they show that there is no striking tendency towards such glycogen deposition and to this extent are in accord with the observations of Cori and Cori which have been mentioned above. In some experiments, the amount of dihydroxyacetone administered was certainly large enough to effect a definite rise in muscle-glycogen provided the triose readily polymerised to form the polysaccharide. In experiment No. 18, in which 9 g. of dihydroxyacetone were administered, a fall of glycogen occurred of 0·425 %, which is by far the greatest fall observed in all experiments, and further, the four experiments in which a fall took place with dihydroxyacetone are those in which the animal received the largest amounts of the triose, and the animal which received the smallest amount of dihydroxyacetone showed the biggest rise. If the correlation coefficient between the amount of triose administered and the change in glycogen be calculated it is found to be $0{\cdot}95 \pm 0{\cdot}02$, which indicates a strong relation between fall in glycogen content and the amount of triose given. As mentioned above, too much emphasis cannot be laid upon the exact figure, because of the comparatively small number of animals used. When the experiments in which dextrose was administered are examined in a similar way, the correlation coefficient is found to be $0{\cdot}56 \pm 0{\cdot}15$. This also indicates the important effect which a large amount of dextrose has in causing the muscle-glycogen to fall. We do not at present propose to discuss the various theories which may be advanced to explain this remarkable result. Since the amount of carbohydrate injected has this effect upon the

disappearance of glycogen from muscle, it is obvious that no conclusion can be drawn, from experiments of this type, regarding the comparative ease with which glycogen can be laid down in muscle.

SUMMARY.

1. When dihydroxyacetone is administered intravenously to decerebrated cats from which the pancreas has been removed, the change in muscle-glycogen depends largely upon the amount of triose administered. With small quantities an increase tends to occur, with large quantities a decrease.

2. When dextrose is administered under similar conditions, results of an analogous type are obtained, although, in this case, the relation between the amount of glucose administered and the change in glycogen may not be so great.

3. The level of the initial blood-sugar, before pancreatectomy, appears to have some effect upon the change in muscle-glycogen, high initial blood-sugars tending to cause an increase. This is probably due to stimulation of insulin secretion by the pancreas.

4. From the experiments, no evidence could be obtained that muscle-glycogen was more readily formed from dihydroxyacetone than from dextrose in presence of the liver.

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CXLI. THE PREPARATION OF TAURINE IN CONSIDERABLE QUANTITY.

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(*Received August 1st, 1927.*)

In order to carry out certain experiments to investigate the action of taurine in its physiological aspects it became necessary to prepare this compound on a considerable scale. Various possibilities of synthesising it were explored but it appeared that in all probability the cheapest and most expeditious method of obtaining the necessary quantity would be to prepare it from some animal product[1], and ox-bile was, under the circumstances, chosen as being most suitable.

Preliminary experiments with fresh ox-bile obtained from the slaughter house, in which the bile was boiled up for eight hours with one-third of its weight of concentrated hydrochloric acid and the product separated from glycine by treatment with alcohol according to the method of Hammarsten [1901] gave yields of 5 g. of taurine per litre of bile. Difficulties were, however, encountered when it was attempted to apply this method on a large scale. For instance it was difficult to obtain three hundred litres of bile from an ordinary slaughter house even over a considerable time, and it was therefore very useful to find that the commercial preparation of sodium tauroglycocholate which can be purchased in large quantities may be used instead of the fresh ox-bile, over which it has many advantages as a source of taurine[2]. It seems, therefore, desirable to put on record the results which have been obtained by the use of this material. Preliminary experiments showed that on a small scale yields of 3–4 g. taurine were obtained from 100 g. sodium tauroglycocholate. On the large scale it was found that 5 kg. of the sodium salt yielded 173 g. taurine. The details of the large scale process follow.

Experimental.

Sodium tauroglycocholate (5 kg.) is dissolved in water (20 l.) and concentrated hydrochloric acid (10 l. $d = 1{\cdot}18$) is added. The mixture is efficiently stirred and boiled under reflux for ten hours. On cooling, the aqueous solution is decanted from the solid hard mass of dyslysin and the latter washed well

[1] Since the work described in this paper was carried out a synthetic method has been described by Marvel, Bailey and Sparberg [1927] which appears capable of giving good yields of taurine from comparatively cheap materials.

[2] It may be noted that in a paper by Rose and Huddleston [1926] in which the effect of taurine on the growth of animals deprived of cystine was investigated references are made to the use of desiccated bile as a source of taurine.

with water. The aqueous solution and washings are evaporated *in vacuo* to about 2 l., and whilst still hot filtered from the sodium chloride (A) which has separated. The filtrate is further concentrated to 300 cc. on the water-bath and again filtered whilst hot (B). The filtrate is diluted with 0·5*N* HCl to 500 cc., poured into absolute alcohol (5 l.), and after standing overnight, the taurine which separates along with some sodium chloride is filtered off, whilst the glycine remains in solution. Recrystallisation of this material from an equal weight of boiling water yields almost pure taurine containing a barely detectable amount of sodium chloride. The compound is washed with absolute alcohol and the alcoholic washings mixed with the filtrate: this precipitates taurine and sodium chloride contained in the mother-liquors. The sodium chloride obtained at the stages A and B (mentioned above) is extracted with a small quantity (about 200 cc.) of boiling water and the solution is filtered hot, the process being repeated if necessary. These filtrates containing taurine, sodium chloride and possibly some glycine are diluted to twice their volume with 0·5*N* hydrochloric acid and the solution is added to ten volumes of absolute alcohol so as to effect separation from the glycine. The precipitated taurine obtained is crystallised from hot water. The mother-liquors from all these crystallisations are evaporated down to a small volume, filtered hot from the sodium chloride which has separated and allowed to cool. The taurine is filtered off and if necessary again crystallised. The combined sodium chloride residues are extracted with boiling alcohol containing potassium hydroxide (2 %) and the hot alkaline extract is neutralised with acetic acid [cf. Abderhalden, 1909]. The comparatively small amount of taurine which separates on cooling is filtered off and purified by recrystallisation from an equal weight of hot water. The total yield of taurine from 5 kg. sodium tauroglycocholate amounts to 173 g.

The alcohol used in the above purification is distilled off and the resulting aqueous solution is evaporated to dryness on the water-bath. The residue is extracted with boiling absolute alcohol, filtered hot, and the filtrate saturated with dry hydrogen chloride. On standing in the ice-chest overnight crystals of glycine ester hydrochloride separate. These are filtered off, washed with a little alcohol, and dried. The yield is 104 g.

The hard mass of dyslysin, which remains after hydrolysing the sodium tauroglycocholate with hydrochloric acid, is not easily attacked by aqueous sodium hydroxide solution but it is soluble in boiling alcoholic potassium hydroxide solution. When the alcohol is removed by distillation in steam an aqueous solution remains from which the bile acids may be precipitated by means of mineral acid. It may be here noted that attempts were made to obtain taurine by alkaline hydrolysis of sodium tauroglycocholate as described by Abderhalden [1909], but on a large scale the considerable quantities of alkali and acid, which must be used, result in the formation of a very large amount of salt and the difficulty of isolating taurine in good yield and in pure condition becomes very great.

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XI

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Certain Quinoline and Benzacridine Derivatives yielding Coloured Adsorption Compounds with Iodine.

By William Ogilvy Kermack, M.A., D.Sc., Robert Henry Slater, B.Sc., Ph.D., and Walter Thomas Spragg.

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MDCCCCXXX.

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XX.—Certain Quinoline and Benzacridine Derivatives yielding Coloured Adsorption Compounds with Iodine.

By William Ogilvy Kermack, M.A., D.Sc., Robert Henry Slater, B.Sc., Ph.D., and Walter Thomas Spragg. (From the Royal College of Physicians' Laboratory, Edinburgh.)

(MS. received June 2, 1930. Read June 2, 1930.)

Introduction.

It has been shown by Barger and Field (1912) and by Barger and Starling (1915) that besides starch certain synthetic organic compounds forming colloidal solutions develop a blue or red colour when treated with a solution of iodine in aqueous potassium iodide. Certain pyrone derivatives have been extensively investigated by Barger and various collaborators, and in the case of certain of these compounds a colour was given by N/100,000 iodine. A colour is given by starch with approximately N/60,000 to N/100,000 iodine, so that certain of these pyrone derivatives are even more sensitive than starch to iodine. The present position of our knowledge regarding these colour reactions has been very completely summarised by Barger in his book on *Organic Chemistry in Biology and Medicine* (1930). The compounds described by Barger and his collaborators are neutral or acidic compounds, and therefore the particles in the colloidal solutions usually carry a negative charge.

It is the purpose of the present communication to describe the colour reactions obtained with certain acridine and quinoline derivatives which are basic in nature, and the particles of which when present in colloidal solution possess a positive charge. The sensitivity of some of these compounds is equal to or greater than that of starch, and so they may be considered as ranking amongst the most active known compounds. In the case of the active acridine derivatives, experiments have been carried out to determine the effect of the variation of the concentration of hydrogen ion, the only ion which appears to have much action on the sensitivity of the reaction. The charge on the colloidal particles, where these were visible under the ultramicroscope, was determined by cataphoresis, whilst in other instances the presumption that they were positively charged was confirmed by precipitation experiments.

1. Derivatives of Benzacridine.

The property of developing a colour in presence of iodine was first observed in the case of 3 : 4-5 : 6-dibenzacridine (fig. 1).

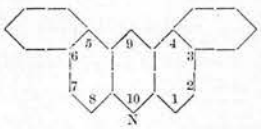


Fig. 1.

The experiments described below were carried out in glassware cleaned in a mixture of sulphuric acid and potassium dichromate, and the distilled water used was of good quality, as shown by the fact that ruby-red gold sols could with ease be made from it.

To 10 c.c. of a 0·05% solution of this compound in absolute alcohol are added 90 c.c. of water with vigorous stirring, when a colloidal solution is obtained which may remain stable for some days. When examined under the ultramicroscope it shows the presence of a large number of small particles in rapid Brownian motion. Experiments to determine the minimum concentration of various salts required to bring about flocculation of the colloid were not very successful because of the somewhat erratic nature of the results obtained. Spontaneous flocculation of the colloid frequently occurs within twenty-four hours, and the exact conditions of its preparation appear to have very considerable influence upon its stability. It was therefore impossible to draw definite conclusions from these experiments about the charge on the particles, but observations in an ultramicroscope in the presence of an electric field showed that the particles in an electrolyte-free medium migrated comparatively rapidly towards the negative pole, and that they therefore possess a positive charge. In presence of very small concentrations of potassium ferricyanide this positive charge is decreased in magnitude, and with higher concentrations becomes negative in sign. In one experiment, where an attempt was made to determine the concentration of potassium ferricyanide in presence of which the charge was approximately zero, it was found that this was in the neighbourhood of N/50,000.

It was observed that dibenzacridine gave a bluish colour with dilute solutions of iodine in aqueous potassium iodide solution. A preliminary experiment showed that the delicacy of this reaction in respect to iodine was of the same order as that of starch, *i.e.* approximately N/100,000.

From the sol prepared as described above a series of dilutions was made. A series of dilutions was also prepared from a standard N/20

iodine solution in 2% aqueous potassium iodide. To each of a series of test-tubes was added 1 c.c. of iodine solution of a particular concentration, and then 1 c.c. of a particular concentration of the sol. The results obtained are shown in Table I, in which the concentrations of dibenzacridine and of iodine are the final concentrations after mixing. Readings were taken after twenty minutes, and no further change had occurred after several hours. Certain tubes (marked *) had completely precipitated.

TABLE I.—VARIED STRENGTHS OF DIBENZACRIDINE AND IODINE IN WATER.

Concentration of iodine.	Concentration of dibenzacridine. 0·0025%.	0·00125%.	0·00062%.	0·00031%.	0·00016%.	0·00008%.	0·00004%.
N/512,000	...	...	...	...	...	...	...
N/256,000	...	...	...	B±	B±	...	...
N/128,000	...	...	B±	B±	B±	...	...
N/64,000	B+*	B+*	B+*	B+	B±	...	...
N/32,000	B++*	B+*	B+*	B+	B±	...	...
N/16,000	B+++*	B++*	B++	B++	B+	...	...
N/8,000	B+++*	B++*	B++*	B+	B+	...	...

Average $pH = 6{\cdot}6$. B = Blue.
Colour diminishes in intensity from +++ very strong to ± very weak.

The following experiment shows the result when the pH was approximately adjusted to 3·0. In this case there was added 0·5 c.c. of N/250 hydrochloric acid, 0·5 c.c. of iodine solution of known concentration, and finally 1 c.c. of a particular dilution of the sol. The final concentration of acid in each tube was therefore N/1000. The results are given in Table II.

A third set of readings were taken in which N/25 hydrochloric acid was used so that the final concentration of acid in each tube was approximately N/100. The results are shown in Table III.

In view of the marked effect of the hydrogen-ion concentration upon the colour, it appeared of interest to carry out an experiment in which the

TABLE II.—VARIED STRENGTHS OF DIBENZACRIDINE AND IODINE IN $\frac{N}{1000}$ HCl.

Concentration of iodine.	Concentration of dibenzacridine. 0·0025%.	0·00125%.	0·00062%.	0·00031%.	0·00016%.	0·00008%.	0·00004%.
$\frac{N}{512,000}$	...	...	...	...	...	...	...
$\frac{N}{256,000}$	...	R±	R±	R±	...	...	...
$\frac{N}{128,000}$	...	R+	R+	R+	...	...	...
$\frac{N}{64,000}$	R+	R+	R+	BR+	B±	...	...
$\frac{N}{32,000}$	R++	BR++	BR+	B+	B±	...	...
$\frac{N}{16,000}$	R+++	B++	B+	B+	B±	...	...
$\frac{N}{8,000}$	B+++	B++	B+	B+	...	...	...

Average $pH = 2{\cdot}8$. B = Blue. R = Red. BR = Bluish red.

TABLE III.—VARIED STRENGTHS OF DIBENZACRIDINE AND IODINE IN $\frac{N}{100}$ HCl.

Concentration of iodine.	Concentration of dibenzacridine. 0·0025%.	0·00125%.	0·00062%.	0·00031%.	0·00016%.	0·00008%.	0·00004%.
$\frac{N}{512,000}$	...	...	...	O±	...	...	...
$\frac{N}{256,000}$	...	...	O±	O±	...	...	...
$\frac{N}{128,000}$	O+	R+	R+	R+	BR±	...	...
$\frac{N}{64,000}$	O+	R+	BR+	BR+	B+	B±	...
$\frac{N}{32,000}$	R++	BR++	B+	B+*	B+*	B±*	...
$\frac{N}{16,000}$	BR+++	B++	B++*	B+*	B+*	B+*	...
$\frac{N}{8,000}$	B+++	B+++*	B++*	Br++	Br++	Br++	Br++

Average $pH = 1{\cdot}4$. B = Blue. BR = Bluish red. R = Red. Br = Brown.

iodine and hydrochloric acid were varied whilst the concentration of colloid remained constant. 0·5 C.c. of acid and 0·5 c.c. of iodine solution were each added to a series of tubes, followed by 1 c.c. of colloid containing 1 part dibenzacridine in 20,000, so that the final concentration of dibenzacridine was 1/40,000. The colours obtained after twenty minutes are shown in Table IV, the *pH* being determined by means of the quinhydrone electrode.

TABLE IV.—EFFECT OF HCl ON SENSITIVITY AND COLOURS GIVEN BY 9-METHYL-3 : 4-BENZACRIDINE.

Concentration of HCl.	Concentration of iodine.											
	N/1,000	N/2,000	N/4,000	N/8,000	N/16,000	N/32,000	N/64,000	N/128,000	N/256,000	N/512,000	N/1,000,000	H_2O.
H_2O	GBr	GBr	P	RB	R	R	R	R	R	R	...	...
0·00062 N	R*	R*	P	B⁰	P	RP	O	O	...	...	...	...
0·0025 N	R*	R	RP	RP	B⁰	B	...	...	...	...	...	...
0·01 N	R*	R	P	P	P	P	...	...	...	...	...	...
0·04 N	R*	R	R	P	O	O	...	...	...	...	...	...
0·16 N	R*	R	R	R	R	R	...	...	...	...	...	...
0·625 N	RBr*	RBr*	R*	R*	R*	R	...	...	...	...	...	...
2·5 N	RBr*	RBr*	R*	R*	R*	R	...	...	...	...	...	...

After twenty-four hours the tubes marked * had precipitated, and the tubes marked ⁰ had developed a red colour in place of the original greenish blue. This may be due to their contents becoming slightly more acid on account of absorption of carbon dioxide. In order to extend these experiments to a slightly more alkaline region a series of observations was made in N/80 sodium bicarbonate, N/80 sodium carbonate, and N/80 sodium acetate solutions. The results are shown in Table V.

It will be seen that any one of a whole range of colours can be obtained by the action of iodine on dibenzacridine according to the conditions of the reaction. A greenish-blue colour is observed when the *pH* is in the neighbourhood of 6–7. When the *pH* is somewhat less the colour observed is a pure blue, except when the concentration of iodine is very low, when a red colour is obtained. With still more acid reactions the blue colour gives place to red when the concentration of iodine is greater, and the red zone is followed by an orange one. With very low *pH*'s (in the neighbourhood of 3) a pure blue colour never occurs.

The effect of decreasing the concentration of dibenzacridine is to shift the red zone to more dilute concentrations of iodine whilst the reddish-blue colour is extended. With high concentrations of iodine a brown colour is developed provided the *pH* is not below 3·0 and provided the concentration of dibenzacridine is not too high. No colour at all develops when the concentration of dibenzacridine is below 0·00016%. This may possibly correspond to the true solubility of the dibenzacridine in water.

TABLE V.

Concentration of iodine.

	$\frac{N}{8,000}$	$\frac{N}{16,000}$	$\frac{N}{32,000}$	$\frac{N}{64,000}$	$\frac{N}{128,000}$	$\frac{N}{256,000}$	$\frac{N}{512,000}$	$\frac{N}{1,000,000}$
$\frac{N}{80}NaHCO_3$	GB+++	GB++	GB+	GB+	GB±	...	...	...
$\frac{N}{80}Na_2CO_3$	GB+++	GB+++	GB++	GB+	GB±	...	...	...
$\frac{N}{80}NaC_2H_3O_2$	GB+++	GB++	GB++	GB+	GB±	...	...	...

Barger and his collaborators (*loc. cit.*) found that the presence of various inorganic salts had a marked effect on the development of the colour with iodine in the case of the pyrone derivatives and cholalic acid, the multivalent cations being particularly important and usually increasing the sensitiveness of the reaction. It therefore appeared of some interest to investigate the effect of the presence of various salts on dibenzacridine.

Potassium chloride (0·01N), potassium sulphate (0·025N), and potassium dihydrogen phosphate (0·125N) were without appreciable effects, whilst calcium chloride and lanthanum chloride were without appreciable action up to 0·002N and 0·025N respectively. However, calcium chloride at a concentration of 0·25N decreases the sensitivity, so that the colour is just visible with N/16,000 iodine, whilst with 0·05N lanthanum chloride N/8000 iodine is required to give the colour. The effect of the presence of calcium chloride and lanthanum chloride in considerable concentration was to reduce the reaction to *pH* 4–5, but it will be seen above that the decreased sensitivity cannot be accounted for by the increase of hydrogen ions.

It was considered of interest to prepare a number of related compounds in order to ascertain whether they too possessed the property of giving a colour with iodine. Partly on account of their ease of preparation the following representative compounds were synthesised; 1 : 2-7 : 8-dibenz-

acridine, 1 : 2-5 : 6-dibenzacridine, 9-phenyl-3 : 4-5 : 6-dibenzacridine, and 9-(-3′-4′-methylenedioxyphenyl-)-3 : 4-5 : 6-dibenzacridine and solutions in absolute alcohol were prepared. The concentrations of these were 0·05%, except in the cases of the relatively insoluble 1 : 2-5 : 6-dibenzacridine and 1 : 2-7 : 8-dibenzacridine, when the concentrations were 0·03%. When 90 c.c. of water were added quickly to 10 c.c. of these alcoholic solutions good colloidal solutions were obtained in every case. These were tested with solutions of iodine in aqueous potassium iodide in concentrations ranging from N/100 to N/10,000, but in no instance was any colour observed. It appears, therefore, that although the colour is given by 3 : 4-5 : 6-dibenzacridine it is not given by a number of closely related compounds.

TABLE VI.

	Concentration of iodine.										
	N/4,000	N/8,000	N/16,000	N/32,000	N/64,000	N/128,000	N/256,000	N/512,000	N/1,000,000	N/2,000,000	
3 : 4-5 : 6-dibenzacridine methosulphate.	GB	GB	GB	GB	GB	R	...	...	...	...	0·005 %
9-methyl-3 : 4-benzacridine methosulphate.	R	RB	B	B	R	R	R	R	R	...	0·005 %

It was thought of interest to synthesise a monobenzacridine in order to test it also. The most readily available compound, namely 9-methyl-3 : 4-benzacridine, was prepared. When 90 c.c. of water is added rapidly to 10 c.c. of a 0·05% solution of this compound in absolute alcohol, a milky sol was obtained, which, however, was only stable for a few hours. This gave a marked colour with iodine, and the reaction was found to possess a high degree of sensitiveness. In Table VI the effect of various concentrations of hydrochloric acid on the sensitiveness and the colour of the reaction are shown. The experimental details are the same as in the analogous experiment already described. In this case dilute iodine solution gives rise to red colours even in neutral solution, and the colour may be detected in an iodine concentration of N/512,000 when no acid is present, but small concentrations of acid give rise to considerable decrease in sensitivity.

The pyrone compounds examined by Barger and Starling (1915) contained an oxygen atom in one of the rings, and it is possible that the iodine attaches itself to the molecule by the residual valencies of the oxygen atom. On the other hand, certain highly unsaturated hydro-

carbons, *e.g.* carotin and lycopin ($C_{40}H_{56}$), give coloured adsorption compounds with iodine, and in these there is of course no atom such as oxygen or nitrogen readily capable of forming co-ordinate compounds. The two active acridine derivatives dealt with above both contain nitrogen, but are feebly basic in nature as a result of the attachment to the nitrogen of two aromatic rings. The iodine might react with the compounds through the residual valency of this feebly basic nitrogen atom. A test of this view appeared possible by forming the methosulphate or other metho-salt of the compound so that the nitrogen atom is made fully saturated and also given a positive charge. It would be expected that, if the nitrogen atom played an essential rôle in the development of the colour, these methosulphates would be devoid of the power of giving colours, especially as it has already been shown that small changes in the constitution of the compound may abolish all power of developing a colour, and as, further, we have ascertained that the methosulphate of the inactive 1 : 2-7 : 8-dibenzacridine is inactive. The absence of chromogenic power in the metho-salts would not, on the other hand, conclusively prove that the nitrogen atom plays a rôle in the reaction, as small changes in the constitution may be sufficient to remove the power, apart from specific changes on any particular atom. The methosulphates of 3 : 4-5 : 6-dibenzacridine and 9-methyl-3 : 4-benzacridine were therefore prepared as described below.

The methosulphate of the dibenzacridine was very sparingly soluble in cold water, but when a solution (0·05%) in absolute alcohol was diluted with nine times its volume of water a clear but strongly fluorescing solution was obtained. A series of dilutions of iodine was prepared in the usual way and mixed with an equal volume of the solution of dibenzacridine methosulphate. In the case of methylbenzacridine methosulphate, which is readily soluble in water, a 0·005% aqueous solution was prepared and mixed with equal volumes of the various iodine solutions. The results are shown in Table VI. It will be seen that dibenzacridine methosulphate gives a colour with iodine up to concentrations of N/128,000, whilst methylbenzacridine methosulphate gives a faint colour with N/1,000,000 iodine. As these two methosulphates dissolve to a greater or less extent in water, it appeared possible that a higher degree of sensitiveness might be shown if the test was carried out by adding the alcoholic solution to the iodine solutions. Experiment showed, however, that the sensitiveness was approximately the same by this method as by the other. In view of the ready solubility of the methylbenzacridine methosulphate in water and its very great sensitivity,

it appeared of interest to ascertain the effect of varying the concentration of the compound as well as that of the iodine. An experiment yielded the results shown in Table VII. It will be seen that the sensitivity is practically constant from 0·05% to 0·005%. At concentrations below 0·005% the sensitivity rapidly decreases and no colour is given with iodine when the concentration of benzacridine methosulphate is N/64,000. Comparison of these figures with those obtained with dibenzacridine shows that the latter substance gives the colour at considerably lower concentrations. This is probably due to the fact that the true solubility of the latter compound is very small.

TABLE VII.—EFFECT OF VARIATION OF CONCENTRATION OF 9-METHYL-3 : 4-BENZACRIDINE METHOSULPHATE AND OF IODINE.

Concentration of benzacridine.	Concentration of iodine.									
	N/4,000	N/8,000	N/16,000	N/32,000	N/64,000	N/128,000	N/256,000	N/512,000	N/1,000,000	N/2,000,000
N/2,000	R	RB	B	B	R	R	R	R	R	...
N/4,000	R	RB	B	B	R	R	R	R	R	...
N/8,000	R	RB	B	B	R	R	R	R	R	...
N/16,000	R	RB	B	B	...	...	...	...	...	...
N/32,000	R	...	...	...	...	...	...	...	...	...
N/64,000	...	...	...	...	...	...	...	...	...	...

A few miscellaneous observations may be recorded here. It is well known that the colour developed by iodine and starch disappears on heating. The colour obtained with 3 : 4-5 : 6-dibenzacridine and 6-methyl-3 : 4-dibenzacridine is stable to heating and does not appreciably change at the boiling-point. In the case, however, of both methosulphates the colour completely disappears on heating. When the methosulphate solution is fairly concentrated the colour disappears only in the neighbourhood of the boiling-point. When the heated methosulphate-iodine solutions are cooled the red colour reappears, but usually not throughout the whole liquid but in the form of an apparently amorphous precipitate.

In the case of the solution containing sufficient iodine to give a blue colour in the cold with methylbenzacridine methosulphate, heating results in the colour changing to red and then disappearing. On cooling, an amorphous precipitate is obtained with a somewhat indefinite purple colour.

The following experiment is of some interest and it will be referred to in the discussion. If two equal quantities of a solution of 9-methyl-3 : 4-benzacridine, of a concentration sufficiently high to give a colour, are placed in two test-tubes, and one of them is heated to the boiling-point and rapidly cooled in cold water until the temperature as shown by a thermometer is equal to that of the unheated solution, and equal quantities of potassium tri-iodide solution are added to each tube, the red colour develops almost instantly in the unheated tube but only slowly in that which was heated. After a few minutes the intensity of colour in both tubes is apparently equal. If the iodine is added a few minutes after cooling, the development of colour occurs as in the unheated tube. This experiment shows that the change which occurs on heating, resulting in the loss of chromogenic power, is one which is not immediately reversible on cooling.

The experiments described above have all been carried out with iodine in aqueous potassium iodide. It has been shown that with iodine and starch and other compounds the presence of iodide ions are necessary unless a sufficiently high concentration of some other anion is present. The other inorganic anions are, however, not so active as the iodide ion. When a dilute silver nitrate solution is added carefully to 3 : 4-5 : 6-dibenzacridine or to the methylbenzacridine methosulphate coloured blue by iodine, so as to remove the iodide ions, the blue colour fades. If now to the mixture to which just sufficient silver nitrate has been added to cause loss of colour a very dilute solution of potassium iodide is slowly added from a burette, the blue colour appears again. The colour, therefore, seems to be dependent on the presence of iodide ions. If, however, an excess of iodide is added the colour may not be seen. This is apparently due to the fact that these benzacridine compounds form iodides highly insoluble in excess of iodide ion, so that under these conditions they are precipitated from solution.

2. Derivatives of 2-Methyl-4-Anilino-6-Methoxyquinoline.

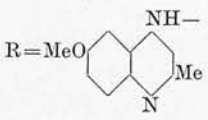
Certain derivatives of 2-methyl-4-anilino-6-methoxyquinoline have been synthesised by one of us (Slater, 1930), and it was observed that in

certain instances their solutions in dilute acetic acid formed gelatine-like gels of considerable rigidity. As the solutions of these compounds apparently existed in colloidal form it was thought that they might yield colours with iodine. When tested, several of them were found to do so. The results are shown in Table VIII.

To carry out the test an 0·05% solution in absolute alcohol was prepared and 90 c.c. of water were added rapidly to 10 c.c. of the alcoholic solution. The almost clear solution which was obtained was then mixed with an

TABLE VIII.—ANILINOQUINOLINE COMPOUNDS IN AQUEOUS ALCOHOL WITH IODINE.

No.	Compound.	Colour Developed.	Minimum Iodine Conc.	Remarks.
1	RC_6H_5	...	...	
2	$RC_6H_4NH_2(3)$. . .	Brown (ppte.)	$\frac{N}{4,000}$	
3	$RC_6H_4NH_2(4)$. . .	Brown . .	$\frac{N}{2,000}$	
4	$RC_6H_4NHCOMe(3)$.	...	...	
5	$RC_6H_4NHCOMe(4)$.	Blue . .	$\frac{N}{8,000}$	
6	$RC_6H_4C_6H_4NHCOMe(4')$	Blue to Red .	$\frac{N}{256,000}$	Blue to $\frac{N}{8,000}$. Reddish blue to $\frac{N}{128,000}$. Red at $\frac{N}{256,000}$.



equal volume of a solution of iodine in aqueous potassium iodide. The concentrations given in the table refer to the final concentrations after addition of the compound. The case of compound 4 calls for special remark. When tested as described above, no colour is developed, and it was considered that a colour might be obtained if a stronger solution was used. 0·1 G. of the compound was added to 10 c.c. alcohol, but complete solution did not take place even on boiling. When, however, 1 c.c. of this alcoholic suspension was added to 9 c.c. of water the compound passed into solution and a clear liquid was obtained. This solution gave no colour when tested in presence of iodine varying from N/500 to N/500,000. If, however, to a series of dilutions of iodine in aqueous potassium iodide solution, 0·1 c.c. of

the alcoholic suspension is added, a blue colour develops immediately in concentrations of iodine up to N/8000. It was noted that the colour developed in the last tube faded completely in about one hour, whilst the intensity in the second last tube gradually diminished. The above facts may possibly be explained as follows. The compound in alcoholic solution is in the anhydrous form, and having a high molecular weight is only slightly soluble even in boiling alcohol. In aqueous alcohol a hydrate is formed and the hydrated molecule would be expected to show a greater affinity for water than the anhydrous compound. Hence the crystals are more readily soluble in aqueous alcohol, and so a true solution is formed which gives no colour with iodine. When, however, the alcoholic solution

TABLE IX.—ANILINOQUINOLINE COMPOUNDS IN ACETIC ACID WITH IODINE.

No.	Compound.	Colour Developed.	Minimum Iodine Conc.	Remarks.
1	RC_6H_5 . . .	...	...	
2	$RC_6H_4NH_2(3)$.	...	...	
3	$RC_6H_4NH_2(4)$.	...	...	
4	$RC_6H_4C_6H_4NH_2(4')$	Blue to bluish red	$\frac{N}{32,000}$	Blue to $\frac{N}{4,000}$.
5	$RC_6H_4NHCOMe(3)$	...	...	
6	$RC_6H_4NHCOMe(4)$	Blue to red	$\frac{N}{64,000}$	Blue to $\frac{N}{4,000}$. Reddish blue to red to $\frac{N}{64,000}$.

R has same significance as in Table VIII.

is added to the water the compound, dissolved in alcohol in its non-hydrated form, is precipitated by the water in a colloidal condition. If iodine is present the complex of iodine with the colloid would be formed and the blue colour would appear. As the colloidal, non-hydrated compound is in an unstable condition, the compound will gradually become hydrated with the disappearance of the blue colour. This will be apparent first of all where the concentration of iodine is least, as under these conditions dissociation of the iodine compound complex will be greatest.

A series of observations was carried out in which the compounds were

first dissolved in 10% acetic acid (0·05 g. in 100 c.c.), and 10 c.c. of these solutions diluted with 90 c.c. of water. Compound 4 is not included in Table VIII as it is highly insoluble in alcohol. The results are given in Table IX.

Four quinoline compounds containing arsenic were also available. These compounds (0·05 gm.) were dissolved in water by the addition of the minimum amount of sodium carbonate, neutralised with N/10 sulphuric acid, and the volume made up to 100 c.c. The milky suspensions obtained were then mixed with an equal volume of iodine solution in the usual way. The results are given in Table X.

TABLE X.—QUINOLINE COMPOUNDS CONTAINING ARSENIC IN WATER WITH IODINE.

No.	Compound.	Colour Developed.	Minimum Iodine Conc.	Remarks.
1	$RC_6H_4AsO(OH)_2(2)$.	Blue . .	$\frac{N}{2,000}$	Colour only given in presence of at least 0·625 N HCl.
2	$RC_6H_4AsO(OH)_2(3)$.	...	...	
3	$RC_6H_4AsO(OH)_2(4)$.	Purple . .	$\frac{N}{8,000}$	
4	$RC_6H_4C_6H_4AsO(OH)_2(4')$	Purple—Red—Brown	$\frac{N}{256,000}$	Purple to $\frac{N}{8,000}$. Red to $\frac{N}{128,000}$.

R has same significance as in Tables VIII and IX.

3. DISCUSSION.

Several points of general interest appear to emerge from the above facts. The compounds previously known to yield colours with iodine form colloidal solutions with water, the particles in many instances being visible under the ultramicroscope. Certain of the compounds which are dealt with here yield typical lyphobic colloidal solutions, and in the case of the derivatives of anilinoquinoline it is clear from the tendency of their solutions to form thick gels that they are probably colloidal in nature, the individual molecules being more or less loosely linked together to form larger aggregates. In the case of another of these compounds evidence has been mentioned above which suggests that in water a molecularly dispersed hydrate is present when equilibrium is established, but that on addition of an alcoholic solution to water the non-hydrated molecules are present in a

colloidal form. These considerations suggest that the anilinoquinoline derivatives probably only show the blue colour with iodine when they exist in solution as aggregates of molecules, and this is confirmed by the fact that in all instances the colour disappears on warming and may reappear on cooling.

The case of the two benzacridine methosulphates is of especial interest. The dibenzacridine methosulphate is very slightly soluble even in cold water, whilst the methylbenzacridine methosulphate is fairly soluble in that solvent. It appears to us highly probable that in solutions of these compounds the degree of dispersion is not molecular but that micelles are present, similar to those known to exist in the soap solutions investigated by McBain. In both cases the molecule consists of a part which by itself would be highly insoluble in water, and the solubility depends on the existence at one point of the molecule of an atom or group possessing an electrical charge and having marked affinity for water. The results of the two opposing tendencies,—that towards aggregation resulting from the hydrophobic nature of the hydrocarbon part of the molecule, and that towards dispersion or solution resulting from the hydrophilic part, leads in the case of the soap solutions to the formation of small colloidal micelles containing aggregates of molecules and also possessing an electric charge, the latter preventing the aggregation of the micelles themselves.

The molecules in a micelle are presumably in equilibrium with a number of molecules in true solution. As the micelle grows the charge on it will increase and the forces opposing the addition of a new charged molecule will become greater. It is therefore to be expected on this view that these benzacridine methosulphates do not exist in solution as separate molecules, but really form highly dispersed colloidal solutions in equilibrium with a certain number of molecules in true solution. It would thus be expected that on warming the coloured benzacridine methosulphate solutions containing iodine the colour would tend to disappear when the concentration of benzacridine methosulphate was low. Dibenzacridine methosulphate is sparingly soluble in water, and the hydrophobic hydrocarbon part of the molecule is bigger than in the case of the methylbenzacridine methosulphate. This compound, therefore, would not be expected to lose its colour with iodine as readily on heating. Experiment shows that this is the case. It is hoped that experiments will be carried out to ascertain whether the above theory as to the constitutions of methylbenzacridine methosulphate in solution is correct.

As the compounds dealt with here in all cases yield organic cations and so form collodial solutions in which the particles are positively charged,

whilst most of the compounds previously known to give a blue colour with iodine form collodial solutions in which the particles are negatively charged, it would appear that the actual charge of the colloidal particles does not play an important rôle in the production of the colour with iodine. In the case of the active, negatively charged colloidal solutions, it has been clearly shown (*cf.* Barger, *loc. cit.*) that the reaction is much facilitated by the presence of multivalent cations. This effect is probably brought about by the influence of these cations on the charge of the colloidal particles and hence on the adsorption of iodine. In the present case the particles themselves are positively charged, and therefore adsorption of iodine should be facilitated if, as in all these experiments, the iodine is present in the form of the negatively charged I_3 ion. It is therefore not altogether surprising that some of these compounds show particularly high sensitivity. Further it is not to be anticipated that the same favourable effect as in the case of negatively charged particles will be brought about by multivalent cations.

It is abundantly clear from the above observations as well as from the previous work of Barger and his collaborators that the production of a blue colour with iodine is a highly specific phenomenon. The adsorption of the iodine on the colloidal particles is apparently a necessary condition, but the actual production of the blue colour appears to be conditioned by the constitution of the molecule. It would appear from the above observations that the whole molecule and not a particular atom or group is concerned. Certain types appear to offer the possibility of the occurrence of the property, but only in particular instances is the property actually developed. Thus of the three isomeric dibenzacridines prepared only one developed the colour. Under these circumstances and until further work has been carried out it appears impossible to advance any satisfactory theory of the reaction.

In conclusion, it may be noted here that some of the compounds dealt with above are even more sensitive to iodine than any previously known. They are comparatively easily prepared and still more sensitive ones may possibly be synthesised. The detection of chlorine in drinking water by the addition of potassium iodide and detection of the iodine liberated by a suitable indicator is of some practical importance, and α-naphthoflavone, investigated by Barger and Starling (1915), and by Hahn, Schütz, and Pavlidés (1928), has been put on the market for this purpose. Certain benzacridine derivatives might prove useful in this direction.

4. Preparation of Benzacridine Derivatives.

3 : 4-5 : 6-Dibenzacridine was prepared according to the method of Mohlau and Haase (1902) by the action of formaldehyde on β-naphthylamine in presence of hydrochloric acid. In most preparations a considerable quantity of "naphthacrihydridine," m.p. 245°, was also obtained which was separated from the dibenzacridine through its very sparing solubility in cold benzene. "Naphthacrihydridine" was readily converted into dibenzacridine by dissolving it in acetic acid and treating it with an equimolecular amount of bromine in acetic acid. The dibenzacridine hydrobromide which separated was filtered off, decomposed with warm dilute alkali, and the base filtered off and recrystallised from benzene; thick, light brown, hexagonal prisms, m.p. 216°.

1 : 2-7 : 8-Dibenzacridine was prepared according to the method of Senier and Austin (1906) by heating α-naphthylamine and methylene chloride in a sealed tube at 220–230°. The product, after treatment with warm alkali and recrystallisation from benzene, formed straw-yellow needles, m.p. 185°.

1 : 2-5 : 6-Dibenzacridine was prepared according to the method of Ullman and Fetvadjian (1903) by heating β-naphthol and α-naphthylamine in presence of paraformaldehyde to 230°. The product, after treatment with alkali, was recrystallised from toluene, when a compound (m.p. 304°) separated, probably analogous to the "naphthacrihydridine" (m.p. 245°) mentioned above. On concentrating the toluene mother liquors, 1 : 2-5 : 6-dibenzacridine separated, and after repeated crystallisation from toluene, formed straw-yellow needles, m.p. 228°.

9-Methyl-3 : 4-benzacridine was prepared according to the method of Ullman and Naef (1900) by adding paraformaldehyde to a mixture of ρ-toluidine and β-naphthol at 200°. When the reaction was complete the product was distilled under ordinary pressure and the fraction (b.p. 300–350°) was collected, treated with warm dilute alkali, and recrystallised from a benzene-ligroin mixture, from which it separated in light brown needles, m.p. 158°.

3 : 4-5 : 6-Dibenzacridine methosulphate was prepared by adding freshly distilled methyl sulphate to 3 : 4-5 : 6-dibenzacridine in hot, dry benzene solution. After refluxing the mixture for fifteen minutes, the yellow crystals which had separated were filtered off and again boiled up in more dry benzene in order to remove any unchanged base. The yellow methosulphate crystallised from alcohol in yellow sheaves of needles, unmelted at 300°. (Found: N, 3·6. $C_{23}H_{19}O_4NS$ requires N, 3·45%.)

3 : 4-5 : 6-Dibenzacridine methosulphate is very sparingly soluble in hot water from which it separates on cooling. It is soluble in ethyl and methyl alcohols and insoluble in benzene, ligroin, and ether. On treating the hot aqueous solution with alkali a white, flocculent deposit separates. Its solution in water or alcohol exhibited a marked fluorescence.

9-Methyl-3 : 4-benzacridine methosulphate was formed when methyl sulphate was added to the base in hot, dry benzene solution. Yellow crystals separated in the hot, which were filtered off and boiled with fresh benzene to remove any unchanged base. On recrystallisation from alcohol it was obtained as light yellow needles, m.p. 205°. (Found: N, 3·9. $C_{20}H_{19}O_4NS$ requires N, 3·8%.)

9-Methyl-3 : 4-benzacridine methosulphate is insoluble in benzene, toluene, and ligroin. It is soluble in water, methyl alcohol, and ethyl alcohol, the solutions exhibiting a strong greenish-blue fluorescence. On the addition of alkali to its solution in water, a white, milky suspension is obtained which gradually flocculates.

9-Phenyl-9 : 10-dihydro-3 : 4-5 : 6-dibenzacridine was prepared according to the method of Claus and Ris (1884), by heating benzaldehyde and β-naphthylamine until steam had ceased to be evolved, and adding β-naphthol, heating being continued at 200° until the reaction is complete. The hot melt is poured into alcohol, and the solid which separated recrystallised from benzene, from which it separates as white, iridescent needles, m.p. 230°.

9-Phenyl-3 : 4-5 : 6-dibenzacridine was prepared by the method of Claus and Ris (1884) by treating the dihydride in benzene solution with an equimolecular amount of bromine in benzene solution. The hydrobromide which separates was decomposed by warming with dilute alkali and the solid filtered off and recrystallised from aniline or toluene, being obtained as white needles, m.p. 293°.

9 : 10-Dihydro-9-(-3′-4′-methylenedioxyphenyl-)-3 : 4-5 : 6-dibenzacridine was prepared by heating piperonal (7·5 g.) and β-naphthylamine (7·2 g.) to 100–110° until no further steam was evolved, and then adding β-naphthol (7·2 g.) and heating to 200–230° until reaction was complete. The hot melt was poured into alcohol. Crystals separated after some time, which were filtered off and crystallised from toluene in light brown prismatic needles, m.p. 305°. (Found: N, 3·8. $C_{28}H_{19}O_2N$ requires N, 3·5%.)

9 : 10-Dihydro-9-(-3′-4′-methylenedioxyphenyl-)-3 : 4-5 : 6-dibenzacridine is insoluble in water and petroleum ether. It is sparingly soluble in alcohol and benzene and quite soluble in toluene and acetic acid. Like

the other acridine derivatives its solution exhibits a striking blue fluorescence.

9-(-3′-4′-methylenedioxyphenyl-)-3 : 4-5 : 6-dibenzacridine was prepared by heating its dihydro derivative in acetic acid solution with an equimolecular amount of bromine. The hydrobromide which separated was filtered off and decomposed by warming with dilute alkali. The base was recrystallised from aniline, when it formed very light yellow prismatic needles, m.p. 282°. (Found : N, 3·4. $C_{28}H_{17}O_2N$ requires N, 3·5%.) It is almost insoluble in alcohol and petroleum ether, slightly soluble in benzene and toluene, and more so in acetic acid, its solutions exhibiting a blue fluorescence.

SUMMARY.

Certain derivatives of benzacridine and of 4-anilinoquinoline give a blue or red colouration with a solution of iodine in aqueous potassium iodide. In the case of the active anilinoquinoline compounds the colour is usually developed in presence of solutions containing iodine at a concentration of the order of N/10,000, whilst in the case of the benzacridine compounds the colour is still apparent at concentrations of N/100,000 or even less. The effect of variation of concentration of compound, iodine, and hydrogen ions has been investigated in certain instances. The action of certain inorganic salts has also been investigated, but in low concentrations these are without much effect. The methosulphates of the two active benzacridine bases also develop colours with iodine even with very low concentrations of the latter. It is suggested that these methosulphates form micellar, colloidal solutions. The chromogenic property appears to depend not on the nitrogen atom in these compounds but on the structure of the molecule as a whole. The compounds which have been investigated differ from most of those previously known to give colours with iodine, in that they are basic and form colloidal solutions in which the particles are positively charged.

We desire to express our thanks to Prof. Barger for his interest and helpful criticism.

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